

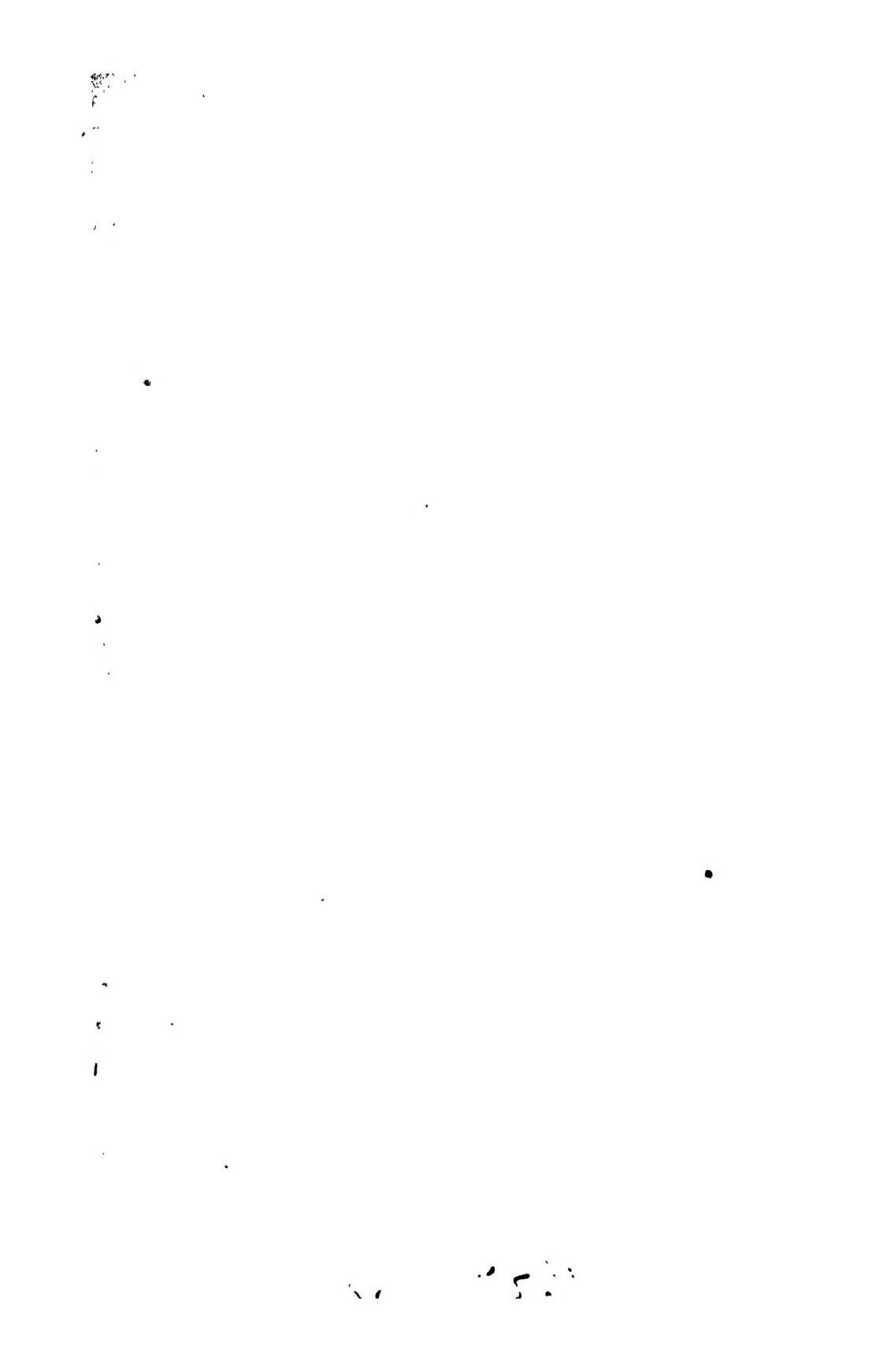


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### HOURLY TRANSPIRATION RATE ON CLEAR DAYS AS DETERMINED BY CYCLIC ENVIRONMENTAL FACTORS

By LYMAN J. BRIGGS, *Biophysicist in Charge, Biophysical Investigations*, and H. L. SHANTZ, *Plant Physiologist, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry*<sup>1</sup>

#### INTRODUCTION

The great differences exhibited by various plants in water requirement—i. e., in the water transpired in the production of a unit of dry matter—are of profound economic importance in the agricultural development of regions of limited rainfall, and an understanding of what gives rise to the greater efficiency which some plants possess in the use of water is highly desirable. The breeding of plant strains adapted to dry-land agriculture has led the writers to undertake a series of transpiration experiments with a view to determining, so far as possible, the influence of various environmental factors on the transpiration rate. To this end simultaneous automatic records have been obtained of the solar-radiation intensity, the depression of the wet-bulb thermometer, the air temperature, the wind velocity, and the evaporation from a free-water surface. The present paper deals with the transpiration response of plants to these factors on clear days.

#### DESCRIPTION OF APPARATUS AND METHODS

##### MEASUREMENT OF TRANSPIRATION

The transpiration measurements described in this paper were carried out at Akron, Colo., in 1912, 1913, and 1914 (Pl. LIII). Transpiration was determined by weighing, four large automatic platform scales of the type already described (Briggs and Shantz, 1915)<sup>2</sup> being used in these measurements. The plants employed were those used in the water

<sup>1</sup> The writers desire to express their indebtedness to the following men for efficient and painstaking assistance in connection with data presented in this paper: Messrs. F. A. Cajori, R. D. Rands, A. MacPhee, J. D. Hird, R. L. Piemeisel, P. N. Peter, H. W. Markward, G. Crawford, and H. Martin.

<sup>2</sup> Bibliographic citations in parentheses refer to "Literature cited," p. 648-649.

requirement investigations, and were grown in the sealed pots already described (Briggs and Shantz, 1913, p. 9), which practically eliminate the direct loss of water from the soil. The pots contained about 115 kgm. of soil and were sufficiently large to enable the plants to make a normal growth, a factor of importance in transpiration measurements (Pl. LV, figs. 1-2). A part of the transpiration measurements were made within the screened inclosure (Pl. LIV, fig. 1) used in the water-requirement experiments to protect the plants from hail and wind storms. Other measurements were made outside the inclosure where the plants were freely exposed, with no protection whatever (Pl. LIV, fig. 2).

#### MEASUREMENT OF PHYSICAL FACTORS

**SOLAR RADIATION.**—The solar-radiation measurements were made automatically with a mechanical differential-telethermograph already described by one of the writers (Briggs, 1913). The instrument has two independent cylindrical bulbs and records only the difference in temperature of the two bulbs. When used for measuring radiation intensity, one bulb is blackened and surrounded by a spherical glass envelope (Pl. LIII). This is so exposed to the sun that the longer diameter of the bulb is normal to the sun's rays at midday. This bulb rises in temperature until the rate at which energy is lost is equal to the rate at which it is received. The other bulb follows the temperature of the air within the instrument shelter, through which the wind blows freely. The instrument was calibrated by comparison with an Abbot silver-disk pyrheliometer (Abbot, 1911). Such comparison shows that the difference in temperature, as measured by the telethermograph, is very nearly proportional to the intensity of the solar radiation falling on a blackened surface normal to the ray, as measured by the pyrheliometer. In other words, the scale is linear and the loss of energy conforms to Newton's law of cooling. While the telethermograph includes the sky radiation as well, the apparatus can be calibrated in terms of the solar radiation on bright days, since on clear days the ratio of sun to sky radiation appears to be fairly constant and the latter at the elevation of Akron (4,200 feet) is small compared with the direct radiation from the sun. A comparison of the telethermograph with the pyrheliometer, when the former is used for measuring radiation, is given in figure 1.

The radiation data given in this paper are expressed in terms of differential temperatures and the mean values are converted to calories per square centimeter per minute on a surface normal to the sun's rays.<sup>1</sup> The radiation is integrated for hourly periods so that zero radiation is not recorded until the hour following the hour interval during which the sun set, or preceding the hour interval during which the sun rose.

<sup>1</sup> The magnification of the differential sunshine instruments was not the same in 1912 and 1914. To convert to calories per square centimeter per minute multiply the differential temperatures in the 1912 observations by 0.0335; and in the 1914 observations by 0.028. In the 1914 observations the instruments were so adjusted as to give differential temperatures in degrees Fahrenheit.

**WET-BULB DEPRESSION.**—The measurement of the depression of the wet bulb was automatically carried on by means of a second differential telethermograph. One bulb was surrounded by muslin which was kept continuously saturated with water by means of a slowly-dripping Mariotte apparatus. In these measurements both bulbs were inside the instrument shelter and protected from solar radiation. The apparatus thus measured the depression of the wet bulb corresponding to the ventilation afforded by the wind through the shelter, which was similar to that to which the plants were subjected.

**EVAPORATION.**—In measuring the evaporation a shallow copper tank 91.3 cm. (3 feet) in diameter and 2.5 cm. deep was used, being mounted on the platform of an automatic scale of the type used in the transpiration measurements. The tank was clamped to a heavy, flat, wooden base,

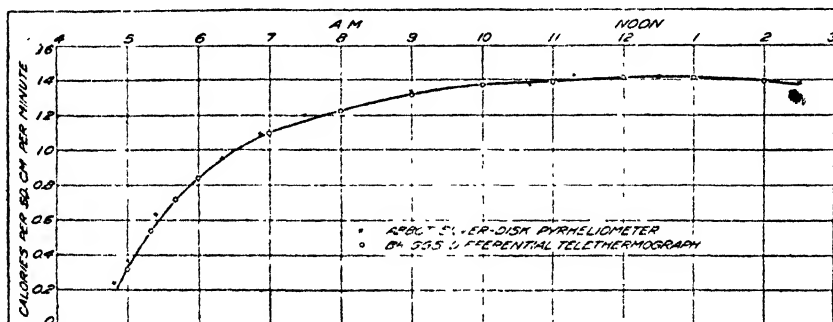


FIG. 1.—Curve showing the comparison of the readings of the differential telethermograph with those of Abbot's silver-disk pyrheliometer.

which was supported on leveling legs about 3 feet above the scale platform (Pl. LV, fig. 3). The inside of the tank was blackened with a mixture of lampblack in "bronzing liquid." The depth of the water in the tank was maintained at approximately 1 cm. by means of a Mariotte apparatus supported from the scale platform and located on the north side of the tank, so that its shadow did not fall on the tank.

**AIR TEMPERATURE.**—The air temperature was measured by a thermograph calibrated with mercurial thermometers and exposed in a standard shelter of the Weather Bureau pattern.

**WIND VELOCITY.**—The wind velocity was measured automatically by an anemometer of the Weather Bureau pattern, located 3 feet above the ground. In the 1914 measurements these measurements were supplemented by a special instrument recording each one-twentieth of a mile.

#### TRANSPIRATION RATE ON CLEAR DAYS IN RELATION TO PHYSICAL FACTORS

The transpiration graph for a single pot of plants for a single day usually shows slight irregularities. In order, therefore, to determine whether such departures are normal or accidental, it is necessary to combine

the transpiration graphs for a number of clear days sufficient to eliminate or minimize the accidental features. In the same manner a composite graph for the corresponding days can be prepared for each of the cyclic environmental factors—radiation, temperature, and wet-bulb depression. The evaporation data have also been combined in the same way. This procedure is not adapted to factors which are essentially noncyclic in character. Wind velocity, for example, is essentially cyclic in some regions and noncyclic in others. While the wind at Akron gives evidence of a daily periodicity, the cyclic character is not sufficiently developed to give the composite graph much weight. The discussion is not, however, limited to the composite values, the hourly values of the transpiration and of each environmental factor being given in the tables for each day considered.

#### WHEAT

The data obtained for the transpiration of wheat (*Triticum* spp.) on clear days in 1912 are given in Table I, and the environmental data for the corresponding period, including solar radiation, air temperature, and wind velocity in Tables II, III, and IV, respectively.

TABLE I.—Transpiration rate (in grams per hour) of wheat, at Akron, Colo., during June and July, 1912

Variety.	Bal- ance No.	Date.	Hour ending—																							
			A. M.												P. M.											
			1	2	3	4	5	6	7	8	9	10	11	Noon.	1	2	3	4	5	6	7	8	9	10	11	12
Turkey	B	June 25	6	6	6	8	10	40	80	140	180	220	240	240	240	270	240	240	220	180	94	30	12	12	10	10
Do	B	26	16	12	14	16	60	110	140	170	170	170	200	210	250	240	200	180	74	14	12	14	12	12	12	
Do	B	27	20	14	8	6	16	20	140	200	240	240	250	260	260	270	250	200	190	46	26	26	14	10	10	
Do	B	28	10	8	8	8	20	110	140	210	270	270	280	280	310	300	240	160	70	20	30	16	12	12		
Do	B	July 5	6	6	12	12	20	60	130	150	170	180	180	180	170	170	140	120	60	22	20	12	12	12		
Do	B	7	14	4	4	4	40	60	140	160	180	170	180	200	220	240	220	190	140	70	20	20	20	20		
Do	B	11	20	16	12	14	2	50	60	160	220	240	280	300	300	260	240	200	100	40	12	20	20	20		
Average for Turkey		13-1	9-4	8-9	9-4	9-7	32-8	88	150	186	210	217	229	237	247	258	219	204	167	73-4	24-5	17-4	14-3	13-7		
Kubanka	A	June 25	4	4	4	4	4	20	40	60	90	100	120	120	120	140	140	130	110	90	40	6	4	4	4	
Do	A	26	4	4	4	4	4	40	56	86	84	100	110	110	120	120	130	120	110	94	46	4	4	4	4	
Do	A	27	4	4	4	4	4	40	80	90	110	100	110	120	140	130	140	130	110	80	20	12	2	2	2	
Do	A	28	4	4	4	4	4	40	20	50	90	100	110	120	150	130	160	160	100	50	10	4	4	4	4	
Do	A	29	4	4	4	4	4	40	70	90	120	140	140	160	170	150	140	130	80	30	14	4	4	4	4	
Do	A	July 5	4	4	4	4	10	32	50	80	120	140	140	160	170	150	160	160	140	50	14	4	4	4	4	
Do	A	7	3	3	3	6	24	60	120	170	160	180	180	200	220	230	200	180	140	50	16	12	6	6		
Do	A	8	3	3	3	6	30	100	140	170	190	220	250	250	260	260	260	250	100	110	16	10	10	10	10	
Average for Kubanka		.....	4-4	4-4	4-4	4-1	5	34-5	65-7	95	212-3	111	141	149	162-5	165	174	160	149	110-5	40-5	11-5	6-5	4-8	4-8	
Khar'kov	C	June 20	4	4	4	4	4	26	60	110	180	240	240	240	270	290	260	260	220	170	80	16	12	12	10	
Do	C	27	18	14	12	12	36	70	180	320	380	380	380	380	380	380	380	380	200	50	30	6	14	10	8	
Do	C	July 7	16	12	8	6	8	12	80	160	280	330	330	340	340	340	340	280	210	180	114	30	10	10	30	
Do	D	June 28	10	10	10	10	10	40	150	220	270	320	330	350	420	420	430	400	240	120	30	20	28	26	18	
Do	D	July 2	4	4	4	4	4	10	120	160	200	230	240	280	320	360	340	250	190	70	24	10	10	12	10	
Do	D	5	5	5	5	5	12	36	60	160	190	230	240	210	200	180	170	140	116	70	26	14	14	14		
Average for Khar'kov		.....	9-5	8-2	7-2	6-8	12-3	35-7	108	193	217	257	255	268	302	300	302	253	183	84	26	15-3	18	17-3	15	
Average, all varieties		.....	8-8	7-1	6-7	6-7	8-7	34-3	85	142-2	170-7	193-3	199	209-5	221-4	231-4	238-1	226-7	192-3	150	67-3	20	12-7	12-8	11-5	
Percentage of maximum		.....	4	3	3	3	4	14	36	60	71	81	84	88	93	97	100	95	81	63	28	8	5	5	4	

TABLE II.—Hourly solar radiation intensity (observed differential temperatures) during wheat transpiration period, at Akron, Colo., during June and July, 1912

Date.	Weight	Hour ending—														
		A M							P. M.							
		5	6	7	8	9	10	11	Noon.	1	2	3	4	5	6	7
June 20	1	5	7	13	16	19	21	21	21	21.5	20	17	14	10	7	0
25	2	5	10	15	19	21	23	24	25	24	24	23	20	11	10	2
26	3	2	10	16	20	21	23	23	25	25	25	24.5	21	18	6	0
27	3	1	10	15.5	18.5	20	21.5	23	25	24	23	23.5	20	15	9	0
28	3	0	18	10	18	21	24	25	25.5	26	26	24	19.5	15	6	2
29	1	3.5	10	15.5	18	20.5	21.5	22	22	22	21.5	20	14	6	1	0
July 2	1	4	10	16	18	21.5	23	23.5	23.5	23	22.5	21	14	7	9	0
5	3	1	5	15	19	21	22	23.5	23.5	23	22.5	20	17	13	9	1
7	3	0	9	15	18	22	24	24	23.5	24	22.5	22.5	17	13	6	0
8	1	0	9	17	18.5	19.5	21	22	22.5	23	21	19.5	18	13.5	8	0
11	1	0	10	15	18	20	21	21.5	22	21	10	11	4	5	6	3.5
Average .		1.6	8.7	15.4	18.4	20.8	22.6	23.6	24.0	23.8	22.7	21.6	17.2	12.7	7.3	.8
Calories per square centimeter per minute																
Percentage of maximum		.05	.29	.52	.62	.70	.75	.79	.80	.80	.76	.72	.58	.43	.24	.03
	7	36	64	77	87	94	98	98	100	99	95	90	72	53	30	3

TABLE III.—Hourly temperatures (in degrees Fahrenheit) during wheat transpiration period, at Akron, Colo., during June and July, 1912

[illegible]



TABLE IV.—Wind velocity in miles per hour during wheat transpiration period at Akron, Colo., during June and July, 1912

Date.	Weight.	Hour ending—																							
		P. M.																							
		Noon.																							
A. M.																									
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 20	1	3.0	3.0	2.0	3.0	3.0	...	...	5	4	3	3.8	3	2.7	2.7	3.1	2.4	1.5	7.6	2	1	1.5	0.8	0.2	1.2
25	2	3.0	2.5	2.0	4.0	5.0	4	7.2	7.5	5	5.5	5.5	4.5	4.5	4.7	5.0	6.0	5.2	2.7	1.5	1.5	1.5	2	3	1
26	3	3.0	6.0	5.2	4.0	3.5	4.5	...	8.6	10.8	13.2	12.8	12.6	10.7	10	9	9.2	3.9	4.1	2.0	1.5	1.5	1	4	4
27	3	1.0	2.5	3.0	3.5	3.0	5.0	...	4.3	4.5	4.0	3.2	3.0	5.5	7.0	6.2	6.7	5.6	7.5	7.5	2.0	3.0	2.0	3.0	4.0
28	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29	1	1.0	1.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
July 2	1	1.0	4.0	2.5	1.5	5.0	5.0	...	4.2	6.0	5.3	2.8	3.0	4.0	4.3	4.0	4.5	7.0	10.5	1.0	7.0	4.0	5.0	2.0	4.0
5	3	8.5	5.0	5.0	1.5	5.0	5.0	...	8.0	8.5	8.0	7.7	7.3	7.0	6.0	4.0	3.7	2.5	2.0	1.5	...	...	...	...	...
7	3	2.0	2.0	5.0	6.0	0.0	4.0	4.3	10.0	9.0	6.5	4.0	3.0	2.7	3.1	6.0	8.0	8.0	7.1	3.0	...	...	...	...	...
8	1	5.0	4.5	7.5	6.0	7.0	9.0	9.3	8.3	5.7	3.7	3.0	2.4	2.2	2.6	2.0	2.9	4.0	6.2	5.5	3.5	1.5	1.0	1.5	2.0
11	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Average		3.3	3.7	3.6	3.1	3.6	4.1	5.7	7.5	7.0	6.7	5.9	5.3	5.1	5.3	5.2	5.7	5.3	5.3	2.8	1.8	1.6	1.8	2.1	2.5

The mean values are plotted in figure 2. It should be recalled that all transpiration measurements in 1912 were made in the hail-screen inclosure (Pl. LIV, fig. 1). The radiation measurements were likewise made under this screen, which reduced the radiation about 20 per cent (Briggs and Shantz, 1914, p. 3). It should also be borne in mind that during the year of 1912 the solar radiation outside the inclosure was about 20 per cent lower than normal (Briggs and Shantz, 1914, p. 54).

The mean solar radiation shown in the first curve of figure 2 is relatively symmetrical, as would be expected if clear or only slightly cloudy days

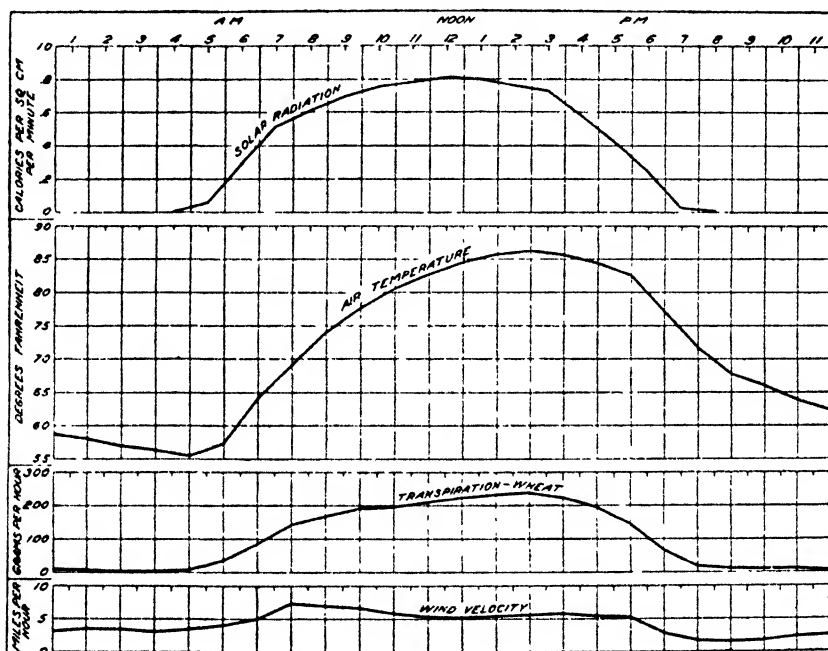


Fig. 2.—Composite transpiration graph of wheat and environmental graphs for corresponding period.

are chosen. The maximum radiation is reached at 12 o'clock, noon, and amounts at that time to only 0.80 calories per square centimeter per minute. The gradient is steep during the early morning and late afternoon, but there is little change in the radiation intensity during the midday hours.

The second graph in figure 2 gives the hourly air temperature in degrees Fahrenheit. The temperature reaches its minimum, 55° F., between 4 and 5 a. m., and its maximum, 86° F., between 2 and 3 in the afternoon. The average temperature from noon to midnight is much higher than from midnight to noon.

The transpiration is recorded in grams per hour. It will be seen from the graph in figure 3 that the transpiration during the night is almost negligible. A marked increase is recorded at 6 o'clock in the morning.

The maximum of 238 gm. per hour is reached about 2.30 p. m., after which the transpiration falls rapidly and acquires the average night rate soon after sunset. There is an indication from the flattening of the curve after 8 o'clock a. m. that from this point on to the maximum the plant modifies its transpiration coefficient.<sup>1</sup> This may be in part due to the closing of the stomata during this period and in part to the lowering of the vapor pressure of the sap of the mesophyll cells resulting from an increase in concentration.

At the bottom of figure 2 is shown the mean wind velocity for each hour of the day. It will be seen that the maximum rate is reached about 7.30 a. m. There is a gradual falling off until about noon, after which the wind velocity remains constant until 5.30 p. m. During the night the rate is somewhat lower.

The transpiration graph of wheat in figure 2 is a composite based upon transpiration measurements of Kharkov and Turkey winter wheats, both

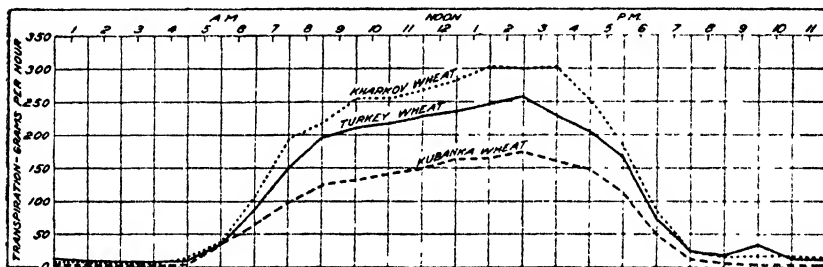


FIG. 3.—Composite transpiration graphs for the three varieties of wheat from which the composite graph of figure 2 was obtained

being varieties of *Triticum aestivum*, and of one hard spring wheat, Kubanka, a variety of *Triticum durum*.<sup>2</sup> The transpiration graphs for each variety, based upon the data given in Table I, are presented in figure 3. It will be noted that the graphs have essentially the same form and that each graph after 9 a. m. shows a falling off in the transpiration rate below that indicated by the slope during the early morning hours.

### OATS<sup>3</sup>

The data covering the transpiration measurements of oats (*Avena sativa*) on clear days are presented in Table V and the environmental measurements for the corresponding period in Tables VI to IX. The

<sup>1</sup> If a plant in its transpiration response to its environment acted as a free physical system, it would be possible to express the transpiration rate in the form of an equation involving the intensity of each of the individual environmental factors. If the relative part played by each factor in determining transpiration were known, then simply by determining the transpiration rate corresponding to some given environment, the transpiration rate for any other environment could be computed. The ratio of the transpiration rate to the environmental intensity would then be defined as the *transpiration coefficient* of the particular plant under observation.

<sup>2</sup> Kharkov, C I (Cereal Investigations) No 1583; Turkey, C. I. No 1571; and Kubanka, C I No 1440.

<sup>3</sup> Swedish Select, C I. No 134

mean hourly values for each environmental factor and for the transpiration are plotted in figure 4. The graphs for the physical factors represent in each case the mean hourly values for eight clear days. The

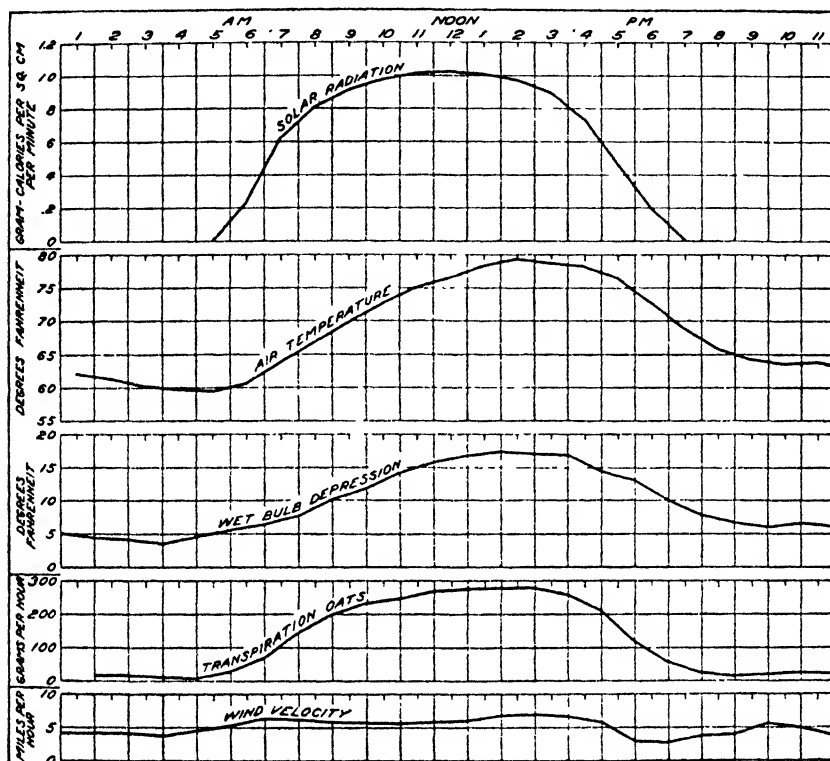


FIG. 4.—Composite transpiration graph for oats, with environmental graphs for corresponding period

transpiration measurements were made in duplicate, using two pots of oats of the same variety, each pot being mounted independently on an automatic balance. The hourly transpiration values plotted in figure 4, therefore, represent the mean of 16 determinations.



TABLE VI.—Hourly solar radiation intensity (observed differential temperature) during oats transpiration period, at Akron, Colo., from August 4 to 18, 1912

Date.	Hour ending—													
	A. M.							P. M.						
	6	7	8	9	10	11	Noon	1	2	3	4	5	6	
Aug. 4.....	7	17	22	26	27	30	30	30	28.5	27	23	7	2	
5.....	7	20	25	28	29	31	31	30	28	26	21.5	17	8	
6.....	7	20	25	28	29	31	31	30	28.5	26	21.5	17	8.5	
7.....	4	17	23	27	29	31	31	30	29	24	19.5	13	2	
8.....	4	16	22	25	27	28	28	28	28.5	26.5	22.5	18	9	
9.....	7	22	25	27	29	30	31	30	29	27	21	3	-1	
10.....	7	20	25	27	29	30	31	30	29	27	22	17	8.5	
11.....	7	20	25	27	29	30	31	30	29	27	22	17	8	
12.....	9	17	25	28	29	31	31	31	29	27	21.5	17	8	
13.....	6	18.4	23.7	26.9	27.5	29.8	30.2	27.7	28.7	26.7	21.5	13.8	5.6	
Average ..	6.6	18.4	23.7	26.9	27.5	29.8	30.2	27.7	28.7	26.7	21.5	13.8	5.6	
Calories per sq. cm. per minute ..	.22	.62	.79	.90	.92	1.07	1.01	1.00	.95	.89	.72	.46	.19	
Percentage of maximum	22	61	78	89	91	99	100	98	95	88	71	46	19	

TABLE VII.—Hourly temperatures (in degrees Fahrenheit) during oats transpiration period, at Akron, Colo., from August 4 to 18, 1912

Date.	Hour ending—													
	A. M.							P. M.						
	1	2	3	4	5	6	Noon	1	2	3	4	5	6	
Aug. 4.....	61.5	61.5	61.5	61.8	62	62	80.6	81	84	81	82.5	78	75	
5.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
6.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
7.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
8.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
9.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
10.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
11.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
12.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
13.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
14.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
15.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
16.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
17.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
18.....	61.5	63	60	61.5	62	65	78	81	81.5	82	81	79	77	
Average ..	62.1	61.3	60.2	59.7	59.3	60.6	70.2	78	79	78.7	78	76.3	72.2	
Average in de- grees centigrade	16.7	16.3	15.7	15.4	15.2	15.9	21.2	25.6	26.1	25.9	25.6	24.6	22.7	
Percentage of maximum range	74	70	5	2	0	7	66	95	100	98	95	86	69	

TABLE VIII.—Hourly wet-bulb depression (in degrees Fahrenheit) during oats transpiration period at Akron, Colo., from August 4 to 18, 1912

Date.	Hour ending—													
	A. M.							P. M.						
	Noon							P. M.						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2
Aug. 4	1	1	1	1	1.5	1.5	2	2	2	2	2	2	2	2
5	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
6	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
7	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
8	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
9	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
10	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
11	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
12	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
13	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
14	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
15	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
16	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
17	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
18	8	9	7	5	4.5	4.5	4.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Average	4.7	4.5	4.2	3.6	4.6	5.6	6.3	7.7	10.1	11.8	14.1	15.6	16.6	17.1
Percentage of maximum	8	7	4	0	7	15	20	30	48	61	78	89	96	100
Saturation deficit	0.140	0.125	0.119	0.095	0.131	0.141	0.159	0.240	0.368	0.392	0.461	0.529	0.584	0.602
Percentage of maximum	23	21	20	16	22	23	31	47	51	65	77	88	97	100

TABLE IX.—Wind velocity (in miles per hour) during oats transpiration period at Akron, Colo., from August 4 to 18, 1912

Date	Hour ending—													
	A. M.							P. M.						
	Noon							P. M.						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2
Aug. 4	10.5	10.2	11.1	7.6	8.3	9.0	7.5	8.0	7.0	7.3	8.5	8.6	8.6	10.2
5	12.1	7.6	6.1	7.0	6.8	5.9	6.0	6.2	8.1	7.2	6.6	6.4	5.6	5.7
6	3.5	3.0	4.4	3.6	5.1	4.5	6.0	5.0	4.2	3.0	3.1	3.5	3.3	4.7
7	3.1	1.05	0	0	0	0	0	2.5	3.0	3.5	3.8	4.7	4.2	6.1
8	4.7	3.9	4.4	7.0	7.8	9.0	8.2	7.2	4.5	4.5	4.5	4.5	4.5	6.5
9	3.7	5.4	5.0	2.0	2.2	2.2	7.3	6.7	9.1	11.0	10.0	10.0	10.0	10.0
10	2.0	2.6	3.4	3.0	2.9	2.9	5.8	5.2	4.4	4.4	4.4	4.4	4.4	8.5
11	1.0	1.1	1.6	2.3	2.9	2.9	5.8	5.4	4.4	4.4	4.4	4.4	4.4	8.5
12	1.2	1.9	1.0	2.9	3.6	4.7	5.1	5.1	3.8	2.8	2.9	3.5	3.5	5.1
Average	4.2	4.1	4.1	3.4	4.4	5.1	6.2	6.0	5.6	5.5	5.4	5.5	5.7	6.6

The smoothness of the graphs obtained by this method of composites is in evidence in figure 4. The radiation curve is again seen to be symmetrical with reference to the noon hour and to decrease in either direction, at first slowly and then rapidly, to zero, a type of curve characteristic of clear days. The air temperature, wet-bulb depression, and transpiration all reach their maximum about two hours later. The transpiration graph for oats, like that for wheat, gives evidence of a slight depression or undue flattening after 9 a. m. In other words, one would expect from the corresponding slopes of the radiation and temperature curves that the transpiration graph would be more convex through the period from 9 a. m. to 2 p. m., provided the oat plant responds as a free physical system. It will be noted that the transpiration rate also falls off more rapidly in the afternoon than does the air temperature or the wet-bulb depression. In this respect the transpiration graph corresponds rather strikingly with the solar-radiation and wind-velocity graphs. The increase in wind velocity during the night does not, however, produce a corresponding increase in transpiration. This point will be referred to again. Finally, it is of interest to note that the transpiration loss of oats under Akron conditions during the night hours is extremely small, compared with the loss during the day.

#### SORGHUM

The sorghum transpiration measurements, like those made with wheat and oats, were conducted inside the screened inclosure and include three varieties of *Andropogon sorghum*—namely, Minnesota Amber, milo, and Dwarf milo<sup>1</sup> (Table X).

The environmental measurements for the corresponding period are given in Tables XI to XIV, inclusive.

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<sup>1</sup> Minnesota Amber, A. D. I. 341-13; milo, S. P. I. No. 24960; Dwarf milo, S. P. I. No. 24970.



TABLE X.—*Transpiration rate (in grams per hour) of sorghum, at Akron, Colo., from August 23 to 29, 1912*

Variety.	Balance No.	Date.	Hour ending—																								
			A. M.												P. M.												
			Noon.																								
			1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
Minnesota Amber	B	Aug. 23	10	10	10	10	10	10	72	170	220	340	400	420	460	440	400	330	340	340	340	54	34	20	20	18	22
Do.	B	24	14	20	10	16	14	24	156	242	320	320	340	430	500	420	370	240	240	240	38	20	22	20	18	22	
Do.	B	25	22	20	20	18	28	80	120	200	200	150	216	160	210	264	260	230	210	28	32	28	24	16	20		
Do.	B	26	20	14	14	14	20	60	150	230	294	374	374	374	352	392	340	310	190	114	28	18	10	4	4		
Do.	B	27	4	4	4	4	12	30	100	180	210	460	440	500	520	590	470	370	244	136	54	40	34	20	4		
Do.	B	28	10	10	10	10	10	10	100	176	232	266	310	360	384	396	386	340	246	140	46	22	22	12	12	12	
Do.	B	29	10	10	10	10	10	10	56	168	212	324	392	436	456	434	386	406	102	78	44	34	14	24	24	20	
Milo.	C	25	18	18	18	10	14	16	100	140	180	280	366	380	410	386	444	310	186	96	24	2	2	2	2	2	
Do.	C	26	16	14	14	14	14	40	100	134	226	288	360	380	520	510	460	310	500	46	32	14	2	2	2	2	
Do.	C	27	2	2	2	2	2	36	100	180	240	300	380	520	510	460	310	500	46	32	14	2	2	2	2	2	
Do.	C	28	12	12	10	10	10	10	10	170	190	230	274	324	356	364	334	292	216	120	26	30	16	18	12	4	
Do.	C	29	12	12	10	10	10	10	4	32	200	210	280	360	360	340	390	370	270	120	36	40	16	18	12	4	
Dwarf milo.	D	24	16	14	14	10	10	16	52	110	150	210	250	334	336	336	300	260	210	110	20	12	12	12	12	16	
Do.	D	25	16	10	10	10	10	10	36	114	160	234	284	354	336	336	300	260	214	100	18	18	20	24	20	14	
Do.	D	26	2	2	2	2	2	8	50	130	190	260	320	340	380	400	332	264	186	70	44	30	24	20	14	12	
Do.	D	27	2	2	2	2	2	8	20	80	130	200	300	320	330	340	330	264	186	70	44	30	24	20	14	12	
Do.	D	29	12	11	11	10	10	20	72	150	206	252	316	382	405	402	368	307	222	94	35	26	20	17	15	12	
Average.			3	3	3	2	2	5	18	37	51	69	83	94	100	99	91	76	55	23	9	6	5	4	4	3	
Percentage of maximum.																											

TABLE XI.—*Hourly solar radiation intensity (differential temperatures) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912*

Date.	Weight.	Hour ending—													
		A. M.								P. M.					
		6	7	8	9	10	11	12	Noon.	1	2	3	4	5	6
Aug. 23	1	6	19	25	26	28	29	29.5	29.5	29.5	27.5	25	20	18	3
24	2	5	14	24	26.5	29	30	31	31	31	31	27	24	19	10
25	3	7	17	24	27	28.5	30	31	31	31	30.5	28	23	19	6
26	2	6	17	23	26	28.5	30	31	31	31	30.5	26	20	14	4
27	3	8	14	23	27	29.5	31	32	32	32	31	25	18	11	5
28	2	9	16	22	24.5	26.5	29	30.5	30.5	30	28.5	26	21	16	9
29	3	4	21	25	27.5	30.5	32	33	33	33	31.5	29	24	19	2
Average		6.5	16.8	23.7	26.5	29	30.4	31.4	31.4	31.2	30.1	27	21.6	16.4	5.5
Calories per sq. cm. per minute.		.22	.56	.79	.89	.97	1.02	1.05	1.06	1.08	1.01	.90	.72	.56	.28
Percentage of maximum.		21	54	75	84	92	97	100	99	99	96	86	69	53	18

TABLE XII.—Hourly temperatures (in degrees Fahrenheit) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912<sup>1</sup>

Date.	Wright.	Hour ending—																							
		A. M.												P. M.											
		Noon.																							
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Aug. 23.....	1	57	56	55	57	55	60	68	73	78	82	86	89	90	90	91	90	86	80	74	70	64	61	63	65
24.....	2	63	60	59	57	56	63	68	72	76	81	85	87	88	89	87	87	84	76	68	66	65	60	65	66
25.....	3	70	65	58	60	60	64	73	75	80	87	91	93	94	94	95	92	89	80	74	73	75	71	67	66
26.....	2	60	62	63	61	60	67	69	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Average.....	64	61.9	59.1	59.1	58.4	64	70.1	73.7	78.3	84.1	88.1	90.3	91.3	91.7	91.7	90	86.8	78.7	72	70.2	69.8	65.7	65.7	65.8	65.8
Average in degrees centigrade.....	17.8	16.6	15.1	15.1	14.7	17.8	21.2	23.2	25.7	28.9	31.2	32.4	32.9	33.2	33.2	32.2	30.4	25.9	22.2	21.2	21	18.7	18.7	18.8	18.8
Percentage of maximum range.....	17	11	2	2	0	17	35	46	60	60	77	89	96	99	100	100	95	85	61	41	35	34	22	22	22

<sup>1</sup> The thermograph record for this period is incomplete, but the days for which no record is given were similar in character to those recorded, as shown by the following maximum and minimum temperatures:

	°F.	
	Maximum.	Minimum.
Aug. 23.....	92	56
24.....	99	57
25.....	91	58
26.....	90	55

TABLE XIII.—Hourly wet-bulb depression (in degrees Fahrenheit) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912

Date.	Weight.	Hour ending—											
		A. M.						P. M.					
		1	2	3	4	5	6	7	8	9	10	11	12
Aug. 23.....	1 5.5	5	5	5	4.5	4	4.5	4.5	4.5	4.5	4.5	4.5	4.5
24.....	2 10.5	11.5	12	10.5	9	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
25.....	3 11	11.5	12	11	10.5	10	10.5	10.5	10.5	10.5	10.5	10.5	10.5
26.....	2 13	12	10.5	10	11	11	11.5	11.5	11.5	11.5	11.5	11.5	11.5
27.....	3 2.5	12	10.5	10	11	11	11.5	11.5	11.5	11.5	11.5	11.5	11.5
28.....	2 8.5	5	5	5	5	5	5	5	5	5	5	5	5
29.....	3 5	5	4.5	3.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Average.....	7.8	7.5	7.2	6.7	6.3	5.8	5.8	6.6	8.6	15.5	19.1	21.9	24.3
Percentage of maximum range.....	10	8	7	4	2	0	4	13	46	64	77	89	98
Saturation deficit, inches.....	0.222	0.206	0.179	0.167	0.179	0.179	0.178	0.218	0.294	0.565	0.750	0.909	1.020
Percentage of maximum.....	19	18	16	15	16	19	19	26	50	66	80	90	97

TABLE XIV.—Wind velocity (in miles per hour) during sorghum transpiration period, at Akron, Colo., from August 23 to 29, 1912

Date.	Weight.	Hour ending—											
		A. M.						P. M.					
		5	6	7	8	9	10	11	12	1	2	3	4
Aug. 23.....	1	5.0	5.3	7.6	10.3	8.9	8.3	7.4	6.1	5.7	5.4	6.2	5.8
24.....	2	5.4	5.3	6.7	8.1	8.2	6.7	8.1	8.2	7.1	4.5	3.9	3.4
25.....	3	6.2	5.6	7.1	10.1	10.0	8.5	8.7	7.1	5.1	6.1	3.9	3.4
26.....	2	6.7	7.2	7.0	9.0	3.8	4.1	4.6	4.6	6.5	7.2	9.6	9.8
27.....	3	1.3	2.0	2.0	9.0	9.8	10.2	10.8	10.7	10.0	10.0	9.4	6.6
28.....	2	3.5	4.5	5.3	6.9	6.9	10.3	8.4	7.3	5.4	4.9	6.1	6.1
29.....	3	2.5	3.8	3.0	7.2	9.8	9.4	10.6	10.2	10.6	7.7	7.1	8.7
Average.....		4.2	5.3	6.1	8.0	7.9	8.4	8.7	8.1	7.6	6.9	6.5	6.7

The sorghum measurements were made during the latter part and the oat measurements during the first part of August. The amplitude and spread of the radiation curves for the two periods are essentially the same (see figs. 4 and 5). The air temperature during the sorghum period was, however, much higher, the average daily maximum being over  $91^{\circ}\text{F.}$ ,

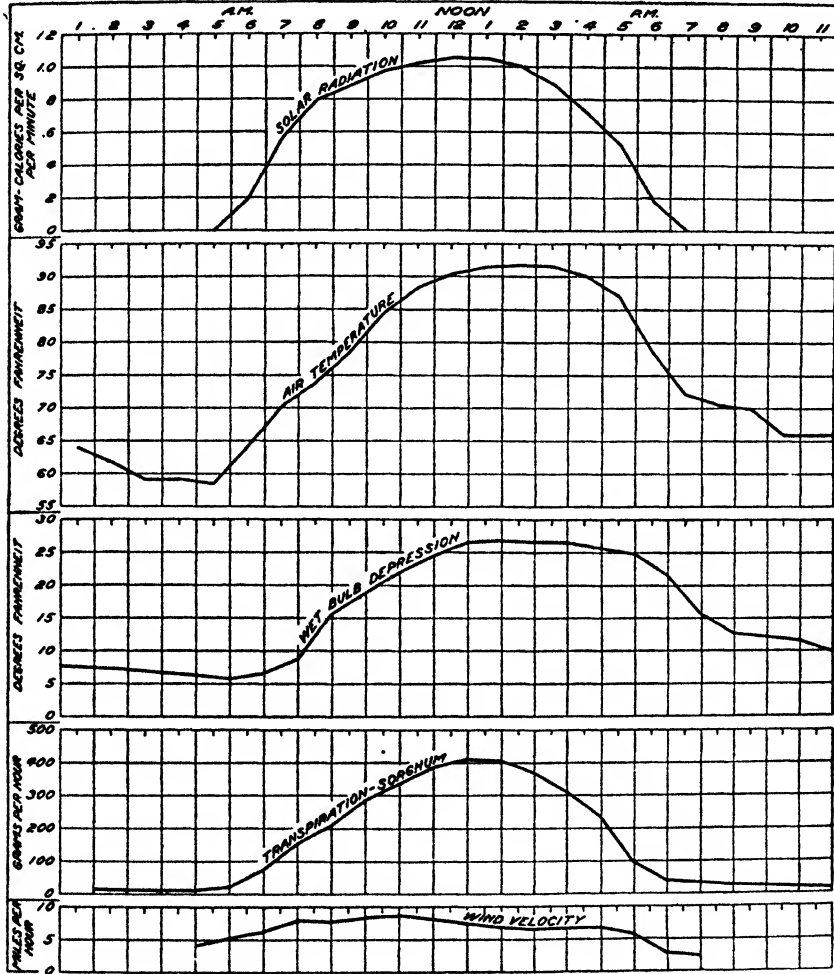


FIG. 5.—Composite transpiration graph of sorghum, with environmental graphs for corresponding period.

compared with a maximum of  $79^{\circ}$  during the oat period. There is also a corresponding difference in the wet-bulb depression, the mean maximum depression during the sorghum period being over  $26^{\circ}$ , compared with  $17^{\circ}$  during the oat period. The conditions were consequently more severe during the sorghum period—i. e., such as to induce a higher transpiration rate. Yet it will be seen, on reference to the transpiration graph

in figure 5, that sorghum, even under the more severe conditions imposed, gave no indication of a flattening of the peak of the transpiration curve. Furthermore, the maximum of the sorghum transpiration curve occurs at approximately noon, and the curve is nearly symmetrical. In brief, the transpiration graph of sorghum appears to follow more nearly the radiation curve than either wheat or oats. It is of interest in this connection to note that sorghum is one of the most efficient of the crop plants in the use of water, the sorghum varieties used in these experiments having a water requirement amounting to only 64 per cent of that of the oat plants.<sup>1</sup>

#### RYE

The transpiration data for rye (*Secale cereale*)<sup>2</sup> on clear days are given in Table XV. These observations were made outside the inclosure, under freely exposed conditions, from June 22 to July 3, 1914. The environmental measurements for this period are given in Tables XVI to XX, inclusive. Hourly evaporation measurements from a free-water surface were also made in 1914, with the aid of an automatic balance. The hourly means for the environmental factors are plotted in figure 6, together with the hourly evaporation and the hourly transpiration of rye, the latter being represented by the mean of 12 automatic records taken on six different days.

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<sup>1</sup> Based upon water-requirement measurements of the same plants. (Briggs and Shantz, 1914.)

<sup>2</sup> Spring rye, C. I. No. 73.

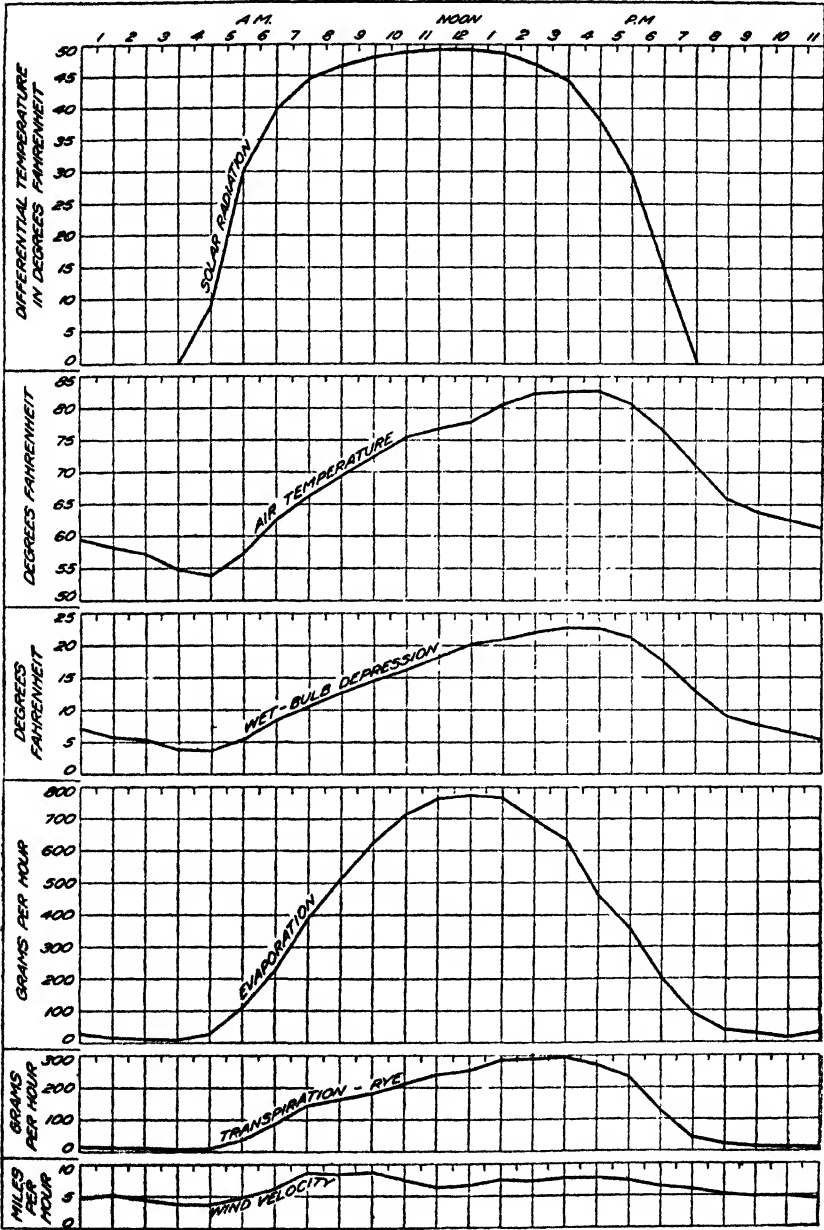


FIG. 6.—Composite transpiration graph of rye, with environmental graphs and evaporation graph for corresponding period.

TABLE XV.—Transpiration rate (in grams per hour) of rye, at Akron, Colo., during June and July, 1914

Date.	Bal- ance No.	Hour ending—												P. M.											
		A. M.												Noon.											
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
June 23.	A					20	80	140	100	100	220	220	260	280	320	300	300	300	230	70	46	30	16	12	12
24.	C					0	100	130	200	210	200	210	230	240	300	300	300	300	200	74	30	10	20	10	10
25.	A	30	40	60	0	0	16	66	100	190	180	200	260	320	300	360	400	350	310	40	40	10	10	0	16
26.	C	20	30	30	0	0	16	66	140	160	200	200	260	300	340	380	360	300	200	60	0	0	0	0	16
27.	A					20	20	100	130	160	184	210	200	210	230	240	260	240	160	40	30	20	20	20	20
28.	C	16	6	0	0	0	6	56	100	128	156	184	210	200	200	240	260	240	160	40	30	20	20	20	20
29.	A					20	10	40	100	120	140	180	180	230	290	280	240	230	140	40	40	10	10	10	10
30.	C	20	0	16	16	20	20	80	140	140	150	150	240	210	200	260	240	230	140	40	40	10	10	10	10
July 1.	A					0	0	20	100	120	140	180	240	240	260	260	240	230	140	40	40	10	10	10	10
2.	C	16	20	10	10	20	50	100	150	190	210	240	240	260	260	260	240	230	140	40	40	10	10	10	10
3.	A					6	16	60	100	130	170	200	200	260	280	300	290	250	200	100	60	20	20	10	10
3.	C					0	16	60	120	140	200	200	200	260	280	290	290	260	200	120	46	36	20	20	0
Average		16	14	14	8	10	38	84	144	166	187	214	240	254	286	290	294	272	234	124	42	22	16	14	12
Percentage of maximum.		5	5	5	3	3	13	29	49	56	64	73	82	86	97	99	100	93	80	42	14	7	5	5	4

TABLE XVI.—Hourly solar radiation intensity (differential temperatures in degrees Fahrenheit) during rye transpiration period at Akron, Colo., in June and July, 1914

Date.	Hour ending—																							
	A. M.												P. M.											
	Noon.																							
	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7		12	1	2	3	4	5	6	7
June 23.	7	18	39	44	48	49.5	50	50	48.5	47	45	43	37	13		50	49	47	45	43	40	37	13	
24.	12	33	42	46	48	50.5	51	50	48.5	47	45	43	40	34	16	48.5	48	46	44	42	39	35	30	28
25.	9	31	39	44	46	47	48	49	48.5	47	45	43	40	34	16	48.5	48	46	44	42	39	35	30	28
26.	7	25	38	43	46	47	48	48.5	48.5	47	45	43	40	34	16	48.5	48	46	44	42	39	35	30	28
27.	11	30	40	44	45	47	48	48.5	48.5	47	45	43	40	34	16	48.5	48	46	44	42	39	35	30	28
28.	10	28	37	45	47	47	48	48.5	48.5	47	45	43	40	34	16	48.5	48	46	44	42	39	35	30	28
Average	9.3	27.5	39.2	44.3	46.7	48.0	48.8	49.1	49.0	48.4	46.7	44.2	38.0	29.3	14.7	49.0	48.4	46.7	44.2	38.0	29.3	24.7	20.3	17.7
Calories per sq. cm. per minute	0.26	0.80	1.10	1.24	1.31	1.34	1.37	1.38	1.37	1.36	1.31	1.24	1.06	0.82	0.41	1.37	1.36	1.31	1.24	1.06	0.82	0.60	0.41	0.30
Percentage of maximum.	19	58	80	90	95	97	99	100	100	99	95	90	77	60	30	100	99	95	90	77	60	40	30	20

TABLE XVII.—Hourly temperatures (in degrees Fahrenheit) during rye transpiration period at Akron, Colo., during June and July, 1914

Date.	Hour ending—													
	A M							P M						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2
June 22.....	64	60	60	57.5	56.5	58.5	61.0	65.0	68.0	69.0	71.0	73.0	74.5	76
24.....	61	61	62	59.0	57.5	61.5	67.5	70.5	74.0	74.5	78.5	81.5	81.0	84
27.....	56	55	54	50.0	49.5	55.0	62.0	65.0	68.5	71.5	75.0	77.0	80.0	81
29.....	58	55	50	48.0	47.5	53.0	59.0	62.0	66.5	70.0	73.0	71.0	70.0	81
July 2.....	57	56.5	56.5	56	56.5	58.5	63.0	67.5	71.5	73.5	76	78	81.5	82.5
3.....	60	61	61	58	56	57	62.5	67	70	73	78.5	80.5	82.5	83
Average in de- greescentigrade	59.3	58.1	57.2	54.8	53.9	57.2	62.5	66.2	69.3	72.3	75.1	76.8	79.8	81.1
Percentage of maximum	15.2	14.5	14.0	12.7	12.2	14.0	16.9	19.0	20.7	22.4	24.1	24.9	26.6	27.3
range.....	19	15	12	3	0	12	30	43	54	64	75	80	90	95

TABLE XVIII.—Hourly wet-bulb depression (in degrees Fahrenheit) during rye transpiration period at Akron, Colo., in June and July, 1914

Date.	Hour ending—													
	A M							P M						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2
June 22.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24.....	7	4	5	3	1	9	9.5	9	11	12	12.5	14	15	16
27.....	12	11	10	7	7	9	12	15	17	18	19	20	21	22
29.....	7	0	5	4	2	3	8	10	12	12	14	17	20	21
July 2.....	5	4	5	4	3	5	7	10	12	12	15	18	21	22
3.....	4	3	3	3	1	7	10	14	17	18	19	21	23	24
Average.....	7.0	5.7	5.4	3.9	3.8	5.4	8.4	10.6	12.8	14.5	16.1	18.2	20.1	20.8
Percentage of maximum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Saturation deficit.....	17	10	8	1	0	8	24	35	47	56	65	76	86	90
Percentage of maximum	22	16	16	12	11	16	32	35	44	54	64	71	83	89





A striking feature of the radiation curve is the rapid rise in radiation intensity during the early morning hours. Reference to the graphs will show that the radiation has attained approximately one-half its maximum value two hours after sunrise, and a corresponding decrease occurs in the late afternoon.

The mean air temperature during the rye transpiration period ranged from 54° F. at 4.30 a. m. to about 83° F. at 4.30 p. m. The maximum air temperature thus occurs four hours later than the solar-radiation maximum. The wet-bulb-depression graph is similar in form to the air-temperature curve, and its maximum occurs at approximately the same time. The maximum of the evaporation curve, on the other hand, corresponds with that of solar radiation, but the slope of the evaporation graph is more nearly uniform during the morning and afternoon than that of the radiation graph.

The transpiration graph of rye shows the same flattening during the middle part of the day that was observed with wheat and oats in 1912. With rye this flattening begins at 8.30 a. m., and continues until 1 p. m., the slope being nearly uniform during this period. During the late afternoon the transpiration falls rapidly and the night transpiration is seen to be very low.

The mean wind velocity in miles per hour is plotted at the bottom of figure 6. The maximum rate of about 9 miles per hour occurs from 8 to 10 o'clock in the morning. During the night the rate is less than 5 miles per hour. There is little indication from the graphs that differences in the velocity of the wind had much influence on either the transpiration or the evaporation rate.

#### ALFALFA

The transpiration measurements upon alfalfa (*Medicago sativa*)<sup>1</sup> are the most extensive of the series and include 52 day records taken during 26 days, embracing late-season as well as midsummer measurements. The transpiration data are given in detail in Table XXI and the physical measurements in Table XXII to XXVI, inclusive. The hourly means will be found plotted in figure 7. Since the period covered by the measurements is so extended, it has seemed advisable also to separate the measurements into shorter periods for comparison. Summaries covering a short transpiration period in June and another period in October are accordingly presented in Tables XXVII and XXVIII, and are plotted in figure 15, to which reference will be made later.

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<sup>1</sup> Grimm alfalfa, A. D. I. (Alkali and Drought Resistant Plant Investigations) No. 23.

TABLE XXI.—*Transpiration rate (in grams per hour) of alfalfa at Akron, Colo., for long periods, in 1913 and 1914*

Date.	Bal- ance No.	Hour ending—												P. M.											
		A. M.						Noon																	
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1913.																									
July 11.....	A	0	0	0	0	60	90	40	110	160	180	160	120	180	220	180	200	190	160	100	30	4	4	4	4
12.....	C	30	20	0	0	20	160	20	90	200	180	200	150	160	200	220	240	260	150	90	4	4	4	4	4
13.....	C	6	6	8	12	20	140	160	160	220	220	220	220	220	240	260	280	240	220	140	50	0	0	0	0
Aug 10.....	C	4	4	4	4	40	20	80	180	200	240	240	180	220	380	400	400	340	200	120	30	10	10	10	10
11.....	C	2	2	2	2	2	20	80	180	240	320	320	340	360	420	400	400	340	260	100	30	4	4	4	4
14.....	C	4	4	4	4	4	20	120	200	300	240	380	400	500	540	600	580	560	480	80	20	14	4	4	4
15.....	C	4	4	4	4	4	20	140	320	400	440	460	520	600	600	600	600	600	440	120	20	10	10	10	10
16.....	C	8	20	20	20	30	70	260	240	300	200	280	280	380	320	340	360	340	240	140	6	6	6	6	10
June 18.....	A	0	0	0	0	20	70	200	200	200	200	280	280	316	324	260	290	250	240	124	36	0	6	6	10
19.....	C	10	10	10	10	10	20	190	220	250	320	360	360	440	460	440	440	440	220	160	160	20	20	0	0
20.....	C	0	0	0	0	0	70	150	220	260	280	300	320	340	380	380	360	350	310	70	70	20	0	0	0
21.....	C	0	0	0	0	0	10	20	220	260	320	400	430	430	460	440	400	400	300	120	66	14	4	0	0
22.....	C	0	0	0	0	40	32	152	260	300	400	460	500	520	540	520	460	360	160	46	10	26	0	0	0
Aug 21.....	A	16	52	80	32	34	100	200	160	300	320	300	440	500	580	580	590	524	479	216	32	34	20	20	32
23.....	C	20	20	60	20	20	10	70	330	420	420	460	560	580	580	580	540	490	430	152	32	20	18	18	22
24.....	C	20	0	0	0	0	0	6	54	280	360	360	440	500	530	560	540	490	430	38	20	0	0	0	6
25.....	C	20	34	10	0	0	20	220	460	620	740	780	840	840	800	760	500	190	30	60	0	0	0	0	0
26.....	C	16	24	0	0	0	0	100	460	560	640	680	720	740	760	710	690	500	188	22	40	10	10	10	6
27.....	C	0	0	0	0	0	0	124	356	540	640	660	680	680	680	670	620	460	200	20	10	10	10	10	6
28.....	C	0	0	0	0	0	0	110	340	450	550	590	620	660	690	750	710	600	428	54	40	20	0	0	0
29.....	C	0	0	0	0	0	0	50	280	400	600	740	780	780	660	660	590	400	60	40	20	0	0	0	0
30.....	C	6	6	6	20	40	20	120	380	480	580	680	700	760	660	660	590	400	100	60	40	10	10	10	6
1914																									
July 1.....	A	0	0	0	0	20	8	120	120	160	350	410	470	454	406	340	360	240	104	32	14	20	10	14	0
2.....	C	0	0	0	0	0	10	88	52	180	228	282	290	320	320	300	340	180	90	24	16	10	14	6	0
3.....	C	12	34	0	0	0	44	116	280	360	430	480	510	480	440	440	380	360	100	34	28	16	12	32	16
4.....	C	10	10	10	10	16	4	80	220	250	330	360	440	410	430	400	360	260	100	20	10	10	16	20	0
5.....	C	20	14	10	10	20	120	380	380	440	580	680	660	710	670	620	620	312	180	28	30	24	16	10	24
6.....	C	14	46	10	16	14	8	100	347	436	514	600	700	780	560	540	500	332	84	36	16	24	16	30	20
7.....	C	16	14	10	20	0	10	100	240	320	340	440	540	520	580	580	540	360	128	24	22	16	30	46	6
8.....	C	6	6	6	20	40	20	120	260	400	640	720	800	840	700	600	680	370	74	12	14	14	26	40	0
9.....	C	10	0	0	10	20	80	240	400	400	510	550	620	640	600	560	500	400	100	60	40	10	10	20	0
10.....	C	0	0	0	0	0	0	72	220	344	420	500	540	560	560	560	500	400	100	20	40	10	10	20	0
11.....	C	0	0	0	14	0	14	60	150	230	330	370	360	340	400	360	360	240	100	20	40	10	10	20	6
12.....	C	0	0	0	14	0	14	60	150	230	330	370	360	340	400	360	360	240	100	20	40	10	10	20	6
1915																									
Oct. 5.....	C	10	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0





TABLE XXIII.—Hourly temperatures (in degrees Fahrenheit) during alfalfa transpiration period at Akron, Colo., for long periods, in 1913 and 1914

Date.		Hour ending—																						
		A. M.						P. M.																
		Noon.																						
1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
1913.																								
July 11.	76	69	61.5	59.5	58	57.5	57.5	59	64	67	71	73	75.5	76	76.5	76.5	76.5	74	71.5	65	61.5	57	54	
12.	49.5	48.5	47.5	49	53	59	66	71	74	77	80	83	85	87.5	88	88.5	87.5	86	73	73	68	68	69	
Aug. 10.	65	64.5	64.5	59	59	60.5	65	70	73	76	78	80	82	83.5	84	83.5	81.5	77	67	73	71	68	65	
14.	63	62	60	59.5	58.5	58.5	63	69	73	78	83	86	89	91	91	92	91.5	91	86	80	76	74	71	
1914.																								
June 18.	59	59	58	58	59.5	60	64	68	72.5	77.5	82	84	86	88	88.5	80	88	84	75	68	65	64	65	
19.	61	62	61.5	61	59	58.5	64	71.5	79	83	85	88	89	90	90.5	90.5	80	88	83	75	73	68	65	
21	61	59	58	57.5	57.5	61.5	67.5	72	75.5	81	83.5	85	87	88	80	86	86	83	77.5	73	68	65	65	
Aug. 11.	60	60.5	61.5	60	58	54	58	62	67	71	73	77	79	80	80.5	80	78	75	67	60	60	60	60	
10.	55.5	55	54.5	52	51	52	56	60	68	71	74	77	80	81	80.5	80	78	75	67	61	53	53	52.5	
18.	61	61.5	62.5	59	58	60	71	80	86	87.5	84	85	85.5	84.5	81	82	76	71	70	68	60	64.5	63.5	
19.	62.5	61.5	60.5	59.5	59	65	72	78	80	83.5	84	85	86	86.5	86	84	79	73	71	63	61	60	60	
20.	58.5	58	57	56	54	54.5	61	69	74	79.5	82	84	86	86.5	80	83	79	73	71	69	68.5	67	64	
23.	42.5	41	41.5	41	39	38	44	52	58	62	67	68	69	70	70.5	68.5	71	72.5	35	40	41.5	41.5	43	
24.	45	44	43.5	40	39	43.5	50	62	67.5	70.5	73	75	76.5	77.5	78	77	74	67	55	52	48	50.5	49	
25.	54.5	54	53.5	52.5	51.5	56	63	71	75.5	77.5	80	81	82	81.5	81	79	74	67	51	48	49	49	49	
26.	57.5	57	55.5	53	49.5	47.5	49	57	67	71	78	81	84	85	85	80	78	72	63	58	58	55.5	55.5	
30.	47	46	47	48	47.5	47	51	62	72	76	80	80.5	80.5	80.5	80.5	80	78	72	64	57	55	52	52	
Oct. 5	37	34	34	34	34.5	35	37	43	53	60	62	64.5	66.5	66.5	66	64	61	58	51	45	43.5	43.5	43	
6	33	31	29.5	30	31	34	42	54	62.5	66	69	71	71.5	71.5	71.5	68	61	54	47	41	40	42	42	
14	23	22.5	22	24	24.5	24	27	34	42	47	51	53	55	58	59.5	59	57	52	44	37	37	36	35	
15	31	31	31	32.5	33	36	41	51	56.5	62	64	66.5	68	69	68	64	59	46	39	35	36	35.5	35.5	
16	36	35.5	36	33.5	33.5	33.5	37	44	54	59	65	69	71	73	74.5	75	73	64	53	47	47	46	45	
17.	45	44.5	44.5	44.5	44.4	44.4	48	56	64	69	73	76	78.5	80	80.5	80	77	65	55	52.5	51.5	51.5	50	
18.	47	40.5	42.5	41.5	38.5	39.5	40	53	60	65	70.5	73	74.5	75	75.5	74.5	70	54	51.5	50	48.5	48.5	47	
19.	44	41	39	38.5	38	36.5	37	49	62	67	70.5	72	73.5	74.5	74	73	69	60	53	47	47	46	45	
20.	44.5	45.5	45	44.5	42.5	40	40	53	67	72	75.5	78	80	81	80.5	78	72	64.5	56	54.5	52.5	51.5	50.5	
Average . . . in de- Average . . . in de- grees centi- grade. Percentage of in a x i m u m range...		51	49.8	49	48.3	47	47.2	50.7	65.7	69.7	73.8	76.1	77.9	79.2	79.7	79.1	77	72	65.6	58.5	56.8	54.6	53.8	
	10.6	9.9	9.4	9.1	8.3	8.4	10.4	14.6	18.7	20.9	23.2	24.5	25.5	26.2	26.5	26.2	25	22.2	18.7	16.4	14.7	12.6	12.1	
	12	9	6	4	0	1	11	34	57	69	82	89	95	98	100	98	92	76	57	45	35	23	21	

TABLE XXIV.—Hourly wet-bulb depression (in degrees Fahrenheit) during alfalfa transpiration period at Atron, Colo., for long periods, in 1913 and 1914

Date.	Hour ending—																							
	A. M.												P. M.											
	Noon.																							
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
<b>1913.</b>																								
July 11.....	10	4.5	3.5	4	4.5	5.2	8	12	14	16	19	20	21	21	21	20.5	20	19.5	17	14.5	12	10	9	7.5
12.....	7	6	5.5	6	7	13	18	20	21.5	23	24.5	26	28	29	29	28	27	24	18	15	14	13	11	
Aug. 10.....	6.5	5.5	4	3	2.5	4	6	8.5	10.5	11	14.5	16.5	19	20	20	22	22.5	21	18	14	11	10	9	
14.....	5	4	3	2.5	2	2	5	9	12	16	17	20	22	24.5	25	26	25	24	18	15	13	11.5	10	
<b>1914.</b>																								
June 18.....																								
19.....	6	4.5	4.5	4	4.5	5	8	10	14	16	20	21.5	24	26	27	25.5	24	20	15	7	5.5	6	8	9
21.....	4	4	4.5	4.5	3.5	6	10	12.5	13	19	24	25	25	25	24	23	22	18	13	12	14	9	7	6
Aug. 11.....	8.5	7.5	6	4.5	4	4	8	9	10	13	19	24	25	25	24	23	22	18	13	12	14	9	7	6
Sept. 10.....	2.5	1	.5	0	0	1.5	3	5.5	8	10.5	13.5	18.5	21	23	24	24.5	25	24.5	19	14	13	10.5	8.5	7
18.....	6	6	6	5	4	4	8	16	21	27	33.5	36	36.5	37.5	37.5	37	36.5	35.5	22.5	16	13	10.5	8.5	7.5
19.....	4.5	3.5	2	2	2	1	2	8	11	17	21	23	26	29	29	27	26	22	17	14	10	8	6	5.5
20.....	5	4	3	3	3	1.5	5	7.5	12.5	17.5	21	23	24	24	24	23	21	19	17	15	12.5	10	8	6
23.....	4.5	3	3	3	3	1	3	7	10	13	15	16	17	17	17	16	15	14	10.5	8	7	4	3.5	
24.....	4.5	4	4	4	4	1	3	11	15	18	20	23	26	27	28	28	27	25	22	17	14	12	11	
25.....	9	10	10	9.5	8.5	8.5	10	15	20	23	26	27	28	28	28	27	25	22	17	14	12	11	11	
26.....	10.5	10	9	7	7	4	5	10	16	18	22	24.5	27	28	28	27	25	22	17	14	12	11	11	
30.....	7	6	6	7	8	7	5	11	16	18	22	24.5	27	28	28	27	25	22	17	14	12	11	11	
Oct. 5.....	1	1	1	1	1	1	1	7	13	16	23	26	28	28	28	27	25	22	17	14	12	11	11	
6.....	3.5	2.5	2	1	1	1	1	8	16	21	26	28	28	28	28	27	25	22	17	14	12	11	11	
14.....	3	1.5	1.5	0	1.5	1.5	1.5	8	16	21	26	28	28	28	28	27	25	22	17	14	12	11	11	
15.....	2.5	1.5	1.5	0	1.5	1	1	8	16	21	26	28	28	28	28	27	25	22	17	14	12	11	11	
16.....	2	2	2	2	1.5	1	1	8	16	21	26	28	28	28	28	27	25	22	17	14	12	11	11	
17.....	7.5	7	7	7.5	7.5	7	7	11	15	19	21	23	23	23	23	22	21	18	14	12	10	8	7	
18.....	10	5	5	3	3	3	3	7	13	21	23	25	27	28	28	26	25	23	19	13	12	10	8.5	
19.....	9	5	5	3	3	3	3	7	13	21	23	25	27	28	28	26	25	23	19	13	12	10	8.5	
20.....	6	5	5	3	3	3	3	7	13	21	23	25	27	28	28	26	25	23	19	13	12	10	8.5	
Average.....	5.8	4.6	4.2	3.8	3.2	3.6	5.3	9.6	13.5	17.3	20.3	22.1	23.6	24.5	25.0	24.3	22.7	19.3	15.0	12.3	10.5	9.0	7.9	6.9
Percentage of maximum.....																								
Range.....	12	6	5	3	0	2	10	39	47	65	78	87	94	98	100	97	89	74	54	43	33	27	22	17
Saturation deficit in inches.....	0.126	0.092	0.091	0.087	0.066	0.075	0.107	0.216	0.372	0.495	0.605	0.685	0.754	0.794	0.827	0.794	0.704	0.572	0.389	0.260	0.218	0.193	0.161	
Percentage of maximum.....	15	11	11	11	8	9	13	27	45	60	73	83	91	96	100	96	86	69	47	37	30	26	23	19

TABLE XXV.—Wind velocity (in miles per hour) during alfalfa transpiration period at Atron, Colo., for long periods, in 1913 and 1914

Date.	Hour ending—											
	A. M.						P. M.					
	1	2	3	4	5	6	7	8	9	10	11	12
1913.	July 11	10	7	7	9	13	12	11	9	11	11	12
	12	10	1	1	3	4	11	12	15	16	14	12
	Aug. 10	10	1	1	3	4	11	12	15	16	14	12
	11	1	2	1	2	1	1	7	5	5	3	2
	14	1	2	1	2	1	1	7	5	5	3	2
1914.	June 18	2	2	3	5	4	5	8	10	8	7	6
	19	1	2	3	5	4	5	8	10	8	7	6
	20	1	2	3	5	4	5	8	10	8	7	6
	Aug. 11	9	10	8	7	6	5	4	3	2	1	0
	12	4	5	6	7	8	9	10	11	12	1	0
	13	4	5	6	7	8	9	10	11	12	1	0
	14	4	5	6	7	8	9	10	11	12	1	0
	15	4	5	6	7	8	9	10	11	12	1	0
	16	4	5	6	7	8	9	10	11	12	1	0
	17	4	5	6	7	8	9	10	11	12	1	0
	18	4	5	6	7	8	9	10	11	12	1	0
	19	4	5	6	7	8	9	10	11	12	1	0
	20	4	5	6	7	8	9	10	11	12	1	0
	21	4	5	6	7	8	9	10	11	12	1	0
	22	4	5	6	7	8	9	10	11	12	1	0
Oct	3	2	2	3	4	5	6	7	8	9	10	11
	4	2	2	3	4	5	6	7	8	9	10	11
	5	2	2	3	4	5	6	7	8	9	10	11
	6	2	2	3	4	5	6	7	8	9	10	11
	7	2	2	3	4	5	6	7	8	9	10	11
	8	2	2	3	4	5	6	7	8	9	10	11
	9	2	2	3	4	5	6	7	8	9	10	11
	10	2	2	3	4	5	6	7	8	9	10	11
	11	2	2	3	4	5	6	7	8	9	10	11
	12	2	2	3	4	5	6	7	8	9	10	11
Average....	4.6	4.6	4.8	4.4	4.3	4.9	5.8	6.8	7.4	8.3	8.2	8.1





TABLE XXVII.—Summary of transpiration and environmental conditions during alfalfa transpiration period at Akron, Colo., from June 18 to 21, 1914

Physical condition.	Hour ending—																								
	A. M.												P. M.												
	1	2	3	4	5	6	7	8	9	10	11	12	Noon.	1	2	3	4	5	6	7	8	9	10	11	12
Transpiration:																									
Average.....	5	8	5	8	17	47	174	227	250	297	347	365	404	414	392	385	365	278	129	64	12	10	2	3	
Percentage of max- imum.....	1	2	1	2	4	11	42	55	60	72	84	88	96	100	96	93	88	67	31	15	3	2	0	1	
Evaporation:																									
Average.....	6	3	3	0	26	100	233	373	540	633	847	867	867	853	607	630	547	300	147	100	80	70	10	60	
Percentage of max- imum.....	0	0	0	0	3	12	27	43	62	73	98	100	100	98	77	73	63	35	17	12	9	8	1	7	
Radiation:																									
Average.....	.....	.....	.....	.....	12	29	39.3	44	45.7	46.3	47.7	48.0	47.0	46.7	44.3	40.0	37.3	28.7	12.0	.....	.....	.....	.....	.....	
Percentage of max- imum.....	.....	.....	.....	.....	25	60	82	92	95	96	100	100	98	97	92	83	76	60	25	.....	.....	.....	.....	.....	
Calories per sq. cm. per minute.....	.....	.....	.....	.....	0.34	0.81	1.10	1.23	1.28	1.30	1.34	1.35	1.32	1.31	1.24	1.12	1.05	0.80	0.34	.....	.....	.....	.....	.....	
Air temperature:																									
Average.....	61.6	60.0	59.1	58.3	57.5	58.6	63.5	69.6	74.5	78.3	82.6	85.1	86.7	88.3	89.0	88.5	88.2	87.3	83.0	76.2	73.0	71.0	66.3	65.0	
Average in degrees centigrade.....	16.4	15.6	15.1	14.6	14.2	14.8	17.5	20.9	23.6	25.7	28.1	29.5	30.4	31.3	31.7	31.4	31.2	30.7	28.3	24.6	22.8	21.7	19.1	18.3	
Percentage of max- imum range.....	13	8	5	3	0	3	19	38	54	66	80	88	93	96	100	98	97	95	81	59	49	43	28	24	
Wet-bulb depression:																									
Average.....	5.0	4.3	4.3	4.3	3.3	5.0	8.7	10.5	12.3	16.0	21.0	22.5	23.7	25.3	25.7	24.5	23.8	20.7	15.0	11.7	12.2	10.0	8.0	5.7	
Percentage of max- imum range.....	8	4	5	4	0	8	24	32	40	57	79	86	91	98	100	95	92	78	52	38	40	30	21	11	
Saturation deficit:																									
Average.....	136	115	125	131	1087	137	143	136	140	1571	1776	1863	1935	1918	1943	1906	1970	1847	1591	1430	1309	1225	1174		
Percentage of max- imum.....	13	11	12	13	8	13	14	14	14	15	17	18	19	20	21	20	21	22	23	24	25	26	27	28	
Wind velocity.....	1.5	2.0	2.5	4.0	3.0	4.0	6.0	7.0	6.7	6.7	7.0	7.0	7.0	6.3	5.3	6.0	7.3	4.0	3.7	2.3	4.2	3.2	2.5	3.5	

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TABLE XXVIII.—Summary of transpiration and environmental conditions during alfalfa transpiration period, at Akron, Colo., from October 16 to 20, 1914

Physical condition.	Hour ending—													
	A. M.							P. M.						
	Noon.													
	1	2	3	4	5	6	7	8	9	10	11	12		
Transpiration:														
Average.....	14	4	6	5	8	21	34	168	269	371	432	468	488	480
Percentage of maximum.....	3	1	1	1	2	4	7	34	55	76	89	96	100	98
Evaporation:														
Average.....	19	18	39	37	25	21	74	150	256	394	572	616	586	544
Percentage of maximum.....	3	3	6	6	4	3	12	24	43	64	93	100	95	88
Radiation:														
Average.....						0	21.0	38.2	41.7	42.5	42.8	43.1	42.3	40.6
Percentage of maximum.....						0	49	89	97	99	99	100	98	94
Calories per sq. cm. per minute.....						0	0.59	1.07	1.17	1.19	1.20	1.21	1.18	1.14
Air temperature:														
Average.....	40.3	41.4	41.4	40.5	39.3	38.7	39.3	49.4	60.8	66.5	70.9	73.6	75.5	76.9
Percentage of maximum.....	4.6	5.2	5.2	4.7	4.1	3.7	4.1	9.7	16.0	19.2	21.6	23.1	24.2	24.9
Percentage of maximum range.....	4	7	7	5	2	0	2	28	58	73	84	91	96	100
Wet-bulb depression:														
Average.....	6.9	4.8	4.6	4.3	3.2	2.8	2.6	8.6	13.8	18.8	21.8	23.6	24.9	25.6
Percentage of maximum range.....	17	9	9	7	3	1	0	26	48	70	83	91	96	99
Saturation deficit:														
Average.....	.129	.090	.086	.081	.061	.054	.053	.184	.348	.508	.627	.704	.745	.783
Percentage of maximum.....	16	11	11	10	8	7	7	21	43	61	77	87	92	96
Wind velocity.....	5.2	4.7	4.9	4.7	4.0	4.9	4.6	5.8	5.7	6.8	6.9	7.6	6.6	6.4

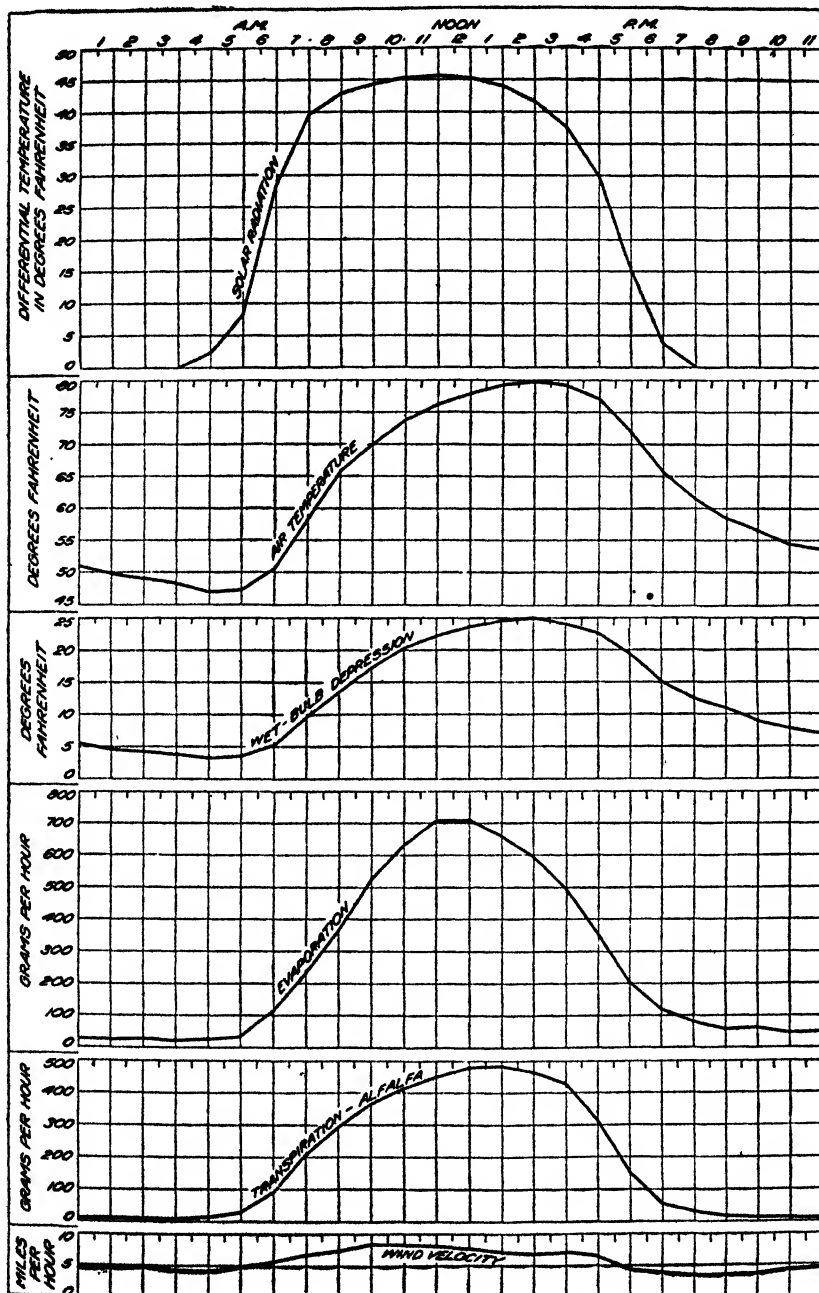


FIG. 7.—Composite transpiration graph of alfalfa, with environmental graphs and evaporation graph for corresponding period.

Considering now the composite graphs based upon the records obtained during 26 clear days, it will be seen that the radiation graph is similar in form to those already discussed, save that the radiation tends to change less rapidly during the early-morning and late-afternoon hours, owing to the fact that the length of the day was not uniform throughout this long period. The slight variation in radiation intensity during the midday hours and the marked changes between 5 and 7 a. m. and 4 and 6 p. m. are in conformity with what has already been noted of the other radiation curves.

The composite temperature graph shows a daily range of 33 degrees, the minimum (47° F.) occurring between 4 and 5 a. m., and the maximum (80° F.) between 2 and 3 p. m. The graph showing the wet-bulb depression is very similar in form to the air-temperature graph, and the maxima and minima correspond. This is to be expected, since with an unvarying amount of water vapor in the air, the wet-bulb depression would be determined by temperature fluctuations. Furthermore, since the observations are confined to clear days, sudden changes in absolute humidity are not encountered.

The evaporation graph representing the alfalfa period is nearly symmetrical with respect to noon, and the slope of the graph changes but slightly during either the morning or afternoon hours. The greater portion of the daily evaporation, however, takes place during the afternoon, owing probably to the higher temperature prevailing during this part of the day.

The transpiration graph shows a very low rate of transpiration during the night. The rate gradually increases from about one hour after sunrise to the maximum at 1.30 p. m. After 2.30 p. m. the curve falls rapidly until sundown and remains practically constant throughout the night. By far the greater part of the daily transpiration occurs during the afternoon. This asymmetry with respect to midday is much more apparent in the transpiration graph than in the evaporation graph.

At the bottom of figure 7 the mean velocity of the wind is shown for each hour in the day. During daylight hours the rate is approximately 7 miles per hour and during the night about 4 miles per hour. It is apparent from Table XXIV that the air is never still for an hour at a time.

#### AMARANTHUS

The transpiration data so far presented have been confined to crop plants. It is also desirable in this connection to study the transpiration of weeds or native plants which have shown themselves adapted to regions of limited rainfall. To this end, *Amaranthus retroflexus* was selected as a plant widely distributed throughout the cultivated areas of the United States. *Amaranthus* is also one of the most efficient plants known as regards the use of water, its water requirement at Akron being below 300, thus comparing favorably with the best of the prosos, millets, and sorghums, the most efficient crop plants known.

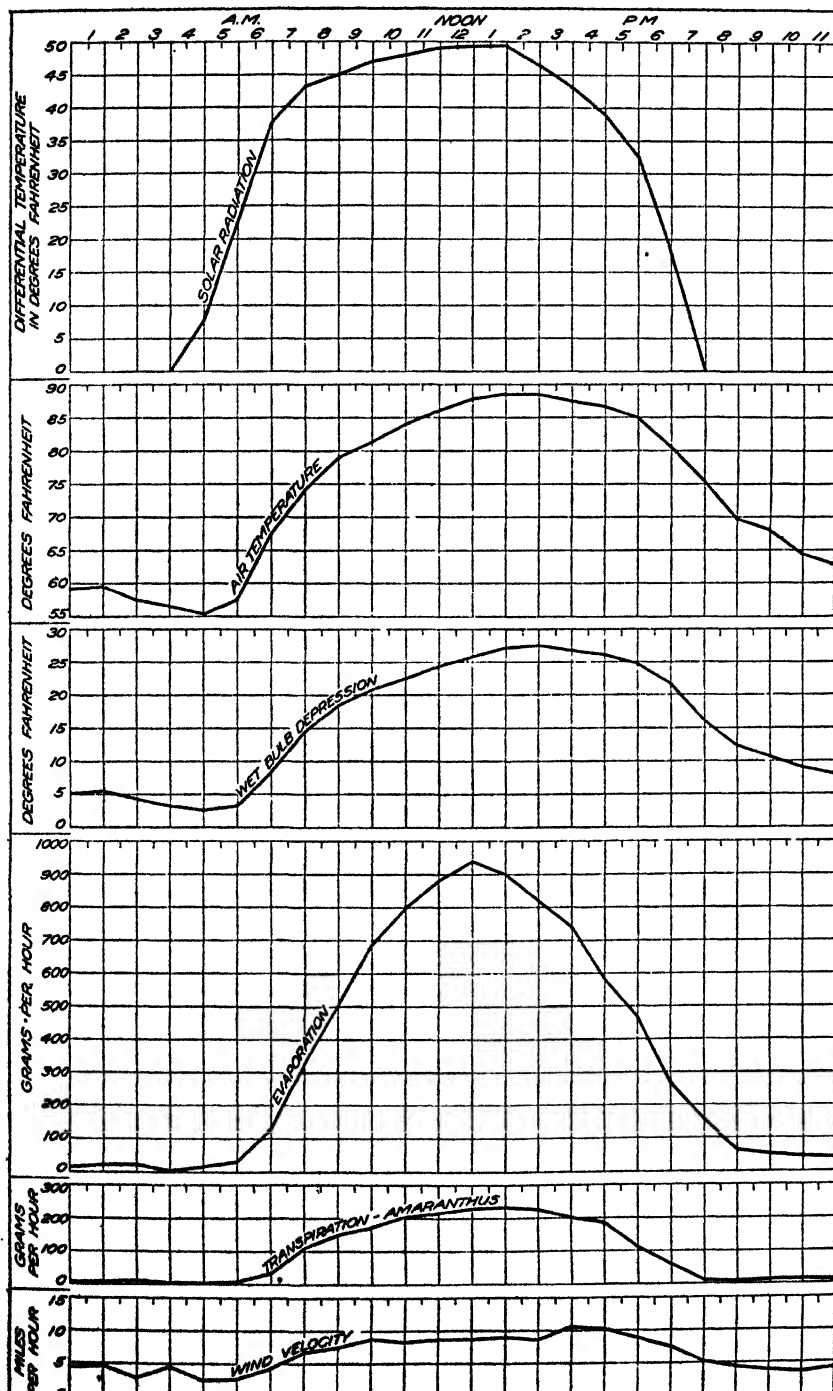


FIG. 8.—Composite transpiration graph for *Amaranthus retroflexus*, with environmental graphs and evaporation graph for corresponding period.

TABLE XXIX.—Transpiration rate (in grams per hour) of *Amaranthus retroflexus* at Akron, Colo., from July 7 to 9, 1914

Date.	Bal- ance No.	Hour ending—													
		A. M.							P. M.						
		1	2	3	4	5	6	7	8	9	10	11	12	1	2
July 7.....	A	0	0	0	0	0	0	15	65	80	90	150	140	168	192
8.....	C	0	0	0	0	6	8	40	74	90	100	180	180	210	220
9.....	C	0	0	0	0	6	8	40	140	180	210	230	230	220	220
.....	A	10	20	20	0	6	10	40	140	200	240	280	280	260	260
.....	C	4	4	20	0	0	10	50	100	200	230	250	260	280	230
Average.....		2	6	10	3	2	6	32	112	152	173	202	215	226	234
Percentage of maximum.....		1	3	4	1	1	3	14	48	65	74	86	92	97	100

TABLE XXX.—Hourly solar radiation intensity (differential temperatures in degrees Fahrenheit) during *Amaranthus retroflexus* transpiration period at Akron, Colo., from July 7 to 9, 1914

Date.	Hour ending—													
	A. M.							P. M.						
	5	6	7	8	9	10	11	Noon	1	2	3	4	5	6
July 7.....	4.0	16	35	42	45	46	47	47	48	50	49	41	34	29
8.....	0.0	23	36	44	44	47	49.0	51	51	49	43	43	42	35
9.....	10.0	29	40	44	45	47	49.0	49	49	49	47	45	41	34
Average.....	7.7	22.7	37	43.3	44.7	46.7	48.3	49.0	49.3	49.3	46.3	43.0	39.0	32.7
Calories per sq. cm. per minute.....	0.21	0.64	1.04	1.21	1.26	1.32	1.34	1.37	1.38	1.38	1.30	1.20	1.09	0.92
Percentage of maximum.....	16	46	75	88	91	95	98	99	100	100	94	87	79	66

TABLE XXXI.—Hourly temperatures (in degrees Fahrenheit) during *Amaranthus* transpiration period at Akron, Colo., from July 7 to 9, 1914

Date.	Hour ending—													
	A. M.							P. M.						
	1	2	3	4	5	6	7	8	9	10	11	Noon.	1	2
July 7.....	60	59	57.5	57.5	56	57	63	68	72	74	78	81	83	84
8.....	58	60	56	54	53	58	70	77	82	85	87	89	91	92
9.....	60	60	59	58	57	58	70	78	83	85	87	88	89	89.5
Average.....	59.2	59.6	57.4	56.5	55.3	57.6	67.7	74.3	79.0	81.3	84.0	86.0	87.7	88.5
Percentage of range.....	15.1	15.3	14.1	13.6	12.9	14.2	19.8	23.5	26.1	27.4	28.9	30.0	30.9	31.4
Percentage of maximum.....	12	13	6	4	0	7	37	57	71	78	87	93	98	100
range.....	12	13	6	4	0	7	37	57	71	78	87	93	98	100

TABLE XXXII.—Hourly wet-bulb depression (in degrees Fahrenheit) during *Amaranthus retroflexus* transpiration period at Akron, Colo., from July 7 to 9, 1914

Date.	Hour ending—													
	A. M.							P. M.						
	1	2	3	4	5	6	7	8	9	10	11	Noon.	1	2
July 7.....	4	4	2.5	3.0	1.0	0.5	4	8	10	11	14	17	19	21
8.....	3	4	4.0	2.0	2.0	3.0	8	17	21	25	26	27	28	29
9.....	8	8	6.0	5.0	5.0	6.0	13	19	24	26	27	29	30	31
Average.....	5.0	5.3	4.2	3.3	2.7	3.2	8.3	14.7	18.3	20.7	22.3	24.3	25.7	27.0
Percentage of maximum.....	9	11	6	2	0	2	23	49	63	73	80	88	94	99
Saturation def. inches.....	0.126	0.137	0.097	0.076	0.067	0.078	0.238	0.498	0.621	0.723	0.823	0.919	0.988	1.051
Percentage of maximum.....	13	13	9	7	6	7	23	47	59	68	78	88	94	100



TABLE XXXIII.—Wind velocity (in miles per hour) during *Amaranthus retroflexus* transpiration period at Akron, Colo., from July 7 to 9, 1914

Date	Hour ending—											
	A. M.						P. M.					
	1	2	3	4	5	6	7	8	9	10	11	12
July 7.....	9	3.8	3	6	3	3	4.7	6.5	8	8	7.5	9
8.....	2.5	7.5	2.5	2.8	2.6	2.2	5	10	8	10	8	8
9.....	2	3	3	5	4	3	3	3.5	6	8	8	8
Average.....	4.5	4.8	2.8	4.6	2.5	2.7	4.2	6.7	7.3	8.7	8.2	8.7

TABLE XXXIV.—Evaporation rate (in grams per hour) during *Amaranthus retroflexus* transpiration period at Akron, Colo., from July 7 to 9, 1914

Date	Hour ending—											
	A. M.						P. M.					
	1	2	3	4	5	6	7	8	9	10	11	12
July 7.....	0	0	0	0	20	40	60	260	460	580	680	800
8.....	0	20	20	0	0	0	220	440	400	760	840	840
9.....	40	40	40	20	20	50	110	260	600	740	800	920
Average.....	13	20	20	7	13	30	130	320	500	694	793	837
Percentage of maximum.....	1	2	2	1	1	3	14	35	54	74	85	94

The transpiration measurements (see Table XXIX) include six day records on three successive days in July. The corresponding physical measurements are given in Tables XXX to XXXIV, inclusive, and the hourly means are plotted in figure 8.

While these measurements were made during what we have termed "clear days," the sky was not wholly free from cumulus cloud during the period, and this is reflected in the radiation curve, which does not quite reach its normal value during the late morning hours.

Comparison with the conditions prevailing during the rye transpiration period, which extended over the two preceding weeks, will show that the evaporation was distinctly higher during the amaranthus period. The temperature during the latter period was slightly lower, but the saturation deficit was greater. Yet the transpiration graph of *Amaranthus retroflexus* gives no indication of the flattening which is so marked in the transpiration graph of rye. There appears then to be a marked difference in this respect in the response of the two plants to the march of radiation and other cyclic factors.

#### GENERAL DISCUSSION

It seems desirable at this point to summarize briefly the prevailing climatic conditions at Akron during the growth period of plants and more particularly during the transpiration periods included in the above determinations (Table XXXV). Akron is located in the rolling short-grass plains of northeastern Colorado. Absolutely clear days seldom occur, but often there are days with only a few light cumulus clouds in the sky, and during such days the plants are rarely shaded from the direct rays of the sun. Such brief interruptions in the direct radiation appear to have little influence on the hourly transpiration rate. On the other hand, there are many days during which cloudiness develops, especially in the afternoon, not infrequently accompanied by light rain and high wind. The number of days which may be classified as clear in the above-defined sense forms consequently a relatively small part of the growth period of the plants. The measurements presented in this paper have been made on practically cloudless days. The radiation intensity at midday on clear days in midsummer is normally about 1.4 calories per square centimeter per minute on a surface normal to the sun's rays. In the 1912 experiments the hazy condition of the atmosphere, together with the shading effect of the hail screen, combined to reduce the maximum radiation to 0.8 calorie during the wheat transpiration period, 1.02 calories during the oat transpiration period, and 1.05 calories during the sorghum measurements. The plants during the 1912 measurements were consequently obliged to dissipate only from 60 to 75 per cent as much solar energy as in the 1914 experiments.

TABLE XXXV.—Summary of plant and environmental data

## CROP OF 1912

	Wheat.			
	Turkey.	Kharkov.	Kubanka.	Mean for all varieties.
Transpiration period.....	June 25 to July 11	June 20 to July 5	June 25 to July 8	June 20 to July 11
Date of cropping.....	Aug. 1	Aug. 1	Sept. 3	.....
Yield of dry matter..... gm.	344	366	270	327
Mean maximum transpiration..... gm. per hour	258	302	174	238
Maximum transpiration..... gm. per hour	320	450	260	.....
Mean maximum radiation, calories per sq. cm. per minute.....	.....	.....	.....	0.8
Mean maximum air temperature..... °F.	.....	.....	.....	86.1
Range in mean wind velocity.....	.....	.....	.....	1.6 to 7.5
Mean maximum transpiration per gram of dry matter harvested.....	0.75	0.83	0.65	0.73

## CROP OF 1912

	Oats.	Sorghum.			
	Swedish select.	Minnesota Amber.	Milo.	Dwarf Milo.	Mean for all varieties.
Transpiration period.....	Aug. 4 to 18	Aug. 23 to 29	Aug. 25 to 29	Aug. 24 to 29	Aug. 23 to 29
Date of cropping.....	Aug. 23	Sept. 26	Sept. 27	Sept. 27	.....
Yield of dry matter..... gm.	411	667	509	434	537
Mean maximum transpiration, gm. per hour.....	271	412	430	354	408
Maximum transpiration..... gm. per hour	464	.....	.....	.....	.....
Mean maximum radiation, calories per sq. cm. per minute.....	1.02	.....	.....	.....	1.05
Mean maximum wet-bulb depression.....	17.1	.....	.....	.....	20.7
Mean maximum saturation deficit, inches.....	0.602	.....	.....	.....	1.138
Mean maximum air temperature..... °F.	79.0	.....	.....	.....	91.7
Range in mean wind velocity.....	2.5 to 6.7	.....	.....	.....	2.4 to 8.7
Mean maximum transpiration per gram of dry matter harvested.....	0.66	0.62	0.84	0.81	0.76

## CROP OF 1914

	Rye.	Alfalfa.			Amaranthus.
		Early period.	Whole period.	Late period.	
Transpiration period.....	June 22 to July 3	June 18 to 21	.....	Oct. 16 to 20	July 7 to 9
Date of cropping.....	July 25	July 11	.....	Oct. 26	July 14
Yield of dry matter..... gm.	186	157	.....	176	122
Mean maximum transpiration, gm. per hour.....	294	414	482	488	234
Mean maximum evaporation, gm. per hour.....	774	867	710	616	940
Mean maximum radiation, calories per sq. cm. per minute.....	1.38	1.34	1.28	1.22	1.38
Mean maximum wet-bulb depression.....	22.8	25.7	25.0	26	27.3
Mean maximum saturation deficit, inches.....	0.820	1.743	0.827	0.811	1.056
Mean maximum air temperature..... °F.	82.6	89	79.7	77	88.5
Range in mean wind velocity.....	3.9 to 8.7	1.5 to 7	3.5 to 8.3	3.8 to 14.4	2.5 to 10.7
Mean maximum transpiration per gram of dry matter harvested.....	1.58	2.64	.....	2.77	1.98

Since transpiration and evaporation are similarly affected by environmental factors, the loss of water from a free-water surface affords a good summation of the intensity of such factors. The total evaporation from a tank 8 feet in diameter with the water surface at ground level at Akron during the months from April to September, inclusive, is 44 inches, based on the records for seven seasons, compared with 33 inches at Dickinson in western North Dakota, 53 inches at Amarillo in the Panhandle of Texas, and 57 inches at Yuma, Ariz. In general, the evaporation increases as one proceeds from north to south through the Great Plains, and the same condition, though less marked, prevails from east to west. The transpiration conditions at Akron are probably as severe as may be found in cultivated areas east of the Rockies in this latitude ( $40^{\circ}$  N.) or to the north of this parallel.

Hourly evaporation measurements with the shallow, blackened tank were not made in 1912. The evaporation rate in 1914 was highest during the amaranthus period, as would be expected from a consideration of the intensity of the environmental factors. The mean maximum evaporation rate for the different periods during the hours near midday ranged from 700 to 900 gm. per hour from a tank of 6,540 sq. cm. in area.<sup>1</sup>

The highest temperatures and the greatest saturation deficits were encountered during the sorghum and amaranthus transpiration periods; yet these conditions produced no flattening of the peak of the transpiration curve of either plant, which is so marked in the case of wheat and rye. The lowest mean temperature and the smallest saturation deficit

<sup>1</sup> A loss of 1,000 gm. from the small tank corresponds to a loss of 0.0386 inch from the 8-foot tank referred to above, based on continuous records for the period, June 16 to September 19, 1914. The large tank loses more slowly during the forenoon, but more rapidly during the night. This is due to the heat capacity of the large tank. The records based on 24-hour periods show good agreement between the two tanks. To those who are more familiar with evaporation as measured by Livingston's atmometer, the following comparison with the shallow blackened evaporation tank used in our experiments will be of interest. The hourly evaporation graph of the porous-cup atmometers does not agree in form with the evaporation graph from the tank. The atmometers show a marked lag during the middle of the day as compared with the evaporation taking place from the tank. This might be anticipated, since the tank receives only the vertical component of the radiation, while the candle type of atmometer receives a smaller percentage of the total radiation at midday in midsummer than earlier or later in the day, due to the vertical walls. The difference is, however, very pronounced even with the new spherical form of porous cup. It is consequently impossible to establish a definite ratio between the evaporation from the Livingston atmometers and the shallow tank used in our experiments. The average ratio may, however, be given. From 6 a. m. to 6 p. m., on August 13 and 14, 1915, an evaporation of 1,000 c. c. from the tank corresponded to an evaporation of 6.5 c. c. from the white candle-type atmometers (1913); of 7.5 c. c. from the same type (1915); of 8.3 c. c. from the white, spherical type (1915); and of 10.9 c. c. from the black candle type (1915). The loss from the atmometers corresponding to 1,000 gm. loss from the shallow tank for different parts of the day is as follows:

Type of atmometer.	6 to 10 a. m.		10 a. m. to 2 p. m.		2 to 6 p. m.	
	6 to 10 a. m.	10 a. m. to 2 p. m.	10 a. m. to 2 p. m.	2 to 6 p. m.	2 to 6 p. m.	6 to 10 a. m.
White candle type (1913).....	7.2	5.1	5.1	8.6	8.6	7.2
White candle type (1915).....	8.2	5.8	5.8	10.0	10.0	8.2
White spherical type (1915).....	9.1	6.7	6.7	10.3	10.3	9.1
Black candle type (1915).....	14.0	8.4	8.4	12.9	12.9	14.0

During the night the atmometers each lost about 3 gm. of water, while the tank showed a slight gain due to deposition of dew. None of these atmometers had ever been used in other measurements, and distilled water was used in all cases. The values given are based on the means of determinations with four atmometers of each type, after the observed evaporation from each atmometer had been multiplied by the standardization coefficient supplied with the apparatus.

occurred during the oat transpiration period. This may account for the fact that the flattening of the transpiration curve of oats is not so marked as in the case of the other cereals.

The wind velocity during these experiments was higher during the daytime than during the night hours. There is a fairly well-defined maximum between 7 and 10 o'clock and another secondary maximum in the afternoon. Wind-still periods seldom occurred.

In Table XXXV are summarized the mean maximum values of the transpiration, evaporation, radiation, saturation deficit, and temperature for each period; and the yield, time of harvest, and the period during which transpiration measurements were made. The range in mean wind velocity and the mean maximum transpiration per gram of dry matter harvested have also been added to the table.

A comparison of the data for the three varieties of wheat shows a close agreement. Kharkov produced the highest yield and transpired at the highest rate. Kubanka produced the least dry matter and transpired at the lowest rate. On the basis of dry matter produced Kharkov transpired most rapidly and Kubanka least rapidly. From a consideration of unpublished data on the transpiration of cereals from seed time to harvest, these observations appear to have been taken during the period of maximum transpiration for the crops considered.

On the basis of transpiration throughout the total period of growth, the relative transpiration of Kharkov and Turkey wheat was the same—i. e.,  $365 \pm 6$  and  $364 \pm 6$  gm. of water, respectively, for each gram of dry matter produced. Kubanka transpired relatively more—i. e.,  $394 \pm 7$  gm. of water for each gram of dry matter.

Oats transpired somewhat less rapidly than wheat in proportion to the amount of dry matter produced. A consideration of the temperature data shows the mean maximum temperature for the oat period to be about 7 degrees lower than for the wheat period. This difference in temperature and the resulting difference in humidity would be sufficient to account for the lower rate of transpiration of oats compared with wheat. On the basis of total transpiration, oats consumed  $423 \pm 5$  gm. of water for each gram of dry matter produced, or 7 per cent more than Kubanka wheat.

Three different varieties of sorghum were used in the transpiration measurements—Minnesota Amber, milo, and Dwarf milo. The plants were apparently at the height of their transpiration during the measurements. The mean maximum transpiration rate of sorghum was higher in proportion to the dry matter harvested than for oats or wheat, but the physical conditions favored a more rapid transpiration during the sorghum period, as is shown by a comparison of the temperature, radiation, and saturation-deficit data. The slope of the sorghum transpiration curve near the peak is also much greater than for either wheat or oats.

The transpiration during the whole period of growth of sorghum, when based on dry matter produced, is practically the same for the three varieties here considered. Minnesota Amber transpired  $239 \pm 2$  gm. of water for each gram of dry matter produced; Dwarf milo,  $273 \pm 4$  gm.; and milo,  $249 \pm 3$  grams.

The transpiration rate of rye, when based on the dry matter harvested, is much higher than for any other crop included in the 1912 water-requirement measurements. This is due in part to the more extreme atmospheric conditions prevailing during this period and in part to the higher water requirement of rye, which is 39 per cent higher than Kunka wheat and 15 per cent higher than Swedish Select oats.

The data presented during the long period for alfalfa were based on plants which yielded different amounts of dry matter. In order to make the comparison more exact, two short periods have been presented. The environmental conditions were somewhat more extreme during the early period, as is shown by a comparison of radiation, temperature, and saturation deficit. The evaporation rate was also higher. On the basis of dry matter harvested, the transpiration during the two periods was the same. It is necessary in this connection to consider the size of the plant at the actual time of the measurements. The late-period crop was harvested 6 days after the period when the transpiration measurements were made, while the early-period crop was harvested 20 days after the termination of the transpiration measurements. It is evident, therefore, that the ratio of transpiration rate to dry matter of the early-period crop would have been considerably higher had this crop been harvested soon after the transpiration measurements were completed.

The most severe environmental conditions in 1914 were encountered during the amaranthus period. Solar radiation was greater and saturation deficit, air temperature, and evaporation higher. On the basis of dry matter, amaranthus transpired less than alfalfa, but more than rye. On the basis of the whole period of growth, the water requirement of amaranthus was much less than rye, the higher rate of transpiration shown in the data here presented being due to the unusually severe conditions prevailing during this period.

While the writers are considering the data in this paper primarily from the standpoint of the relative transpiration rate of the different plants and are not particularly concerned with absolute values, it is interesting to find that the data here presented conform as nearly as can be expected to the relative transpiration rates of the different plants as determined from the water-requirement measurements.

#### COMPARISON OF THE FORM OF THE CURVES

In order that a more accurate comparison may be made between the form of the transpiration graph and that of the several environmental factors, the mean hourly values presented in the preceding tables have

also been expressed in terms of percentage of the maximum. In the case of temperature and wet-bulb depression, the calculation has been based on the maximum range—i. e., the mean minimum is taken as zero on the scale. The data for the various crops reduced to this uniform basis are presented in figures 9 to 15, inclusive, the axis of abscissas representing time and the axis of ordinates the percentage of the mean daily maximum (or mean daily range).

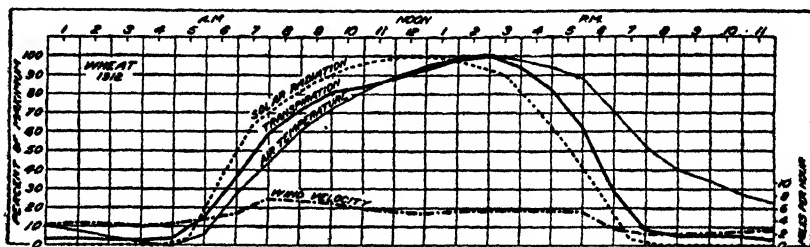


FIG. 9.—Graphs showing transpiration of wheat and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

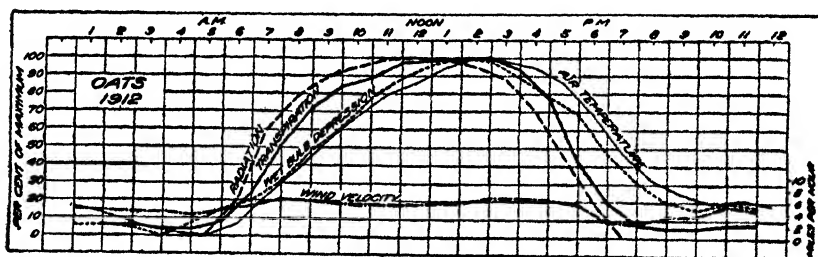


FIG. 10.—Graphs showing the hourly transpiration of oats and the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

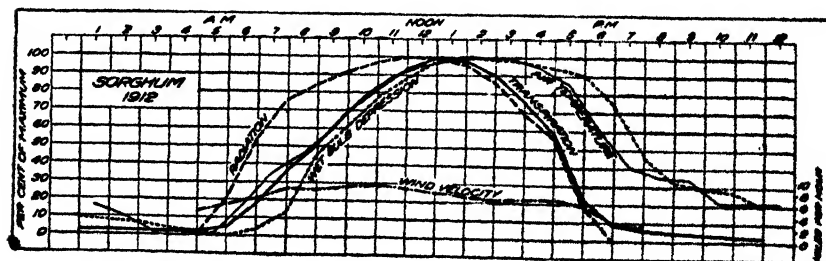


FIG. 11.—Graphs showing the hourly transpiration of sorghum and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

An inspection of the charts will show that the radiation graph rises in advance of the other cyclic environmental factors. This is to be expected, since the change in radiation is the primary cause of the cyclic change of the other components. For the same reason the radiation also rises in advance of the transpiration and falls either in advance of it, as in

the case of the three cereals wheat, oats, and rye, or approximately with the transpiration, as in the case of sorghum, alfalfa, and amaranthus.

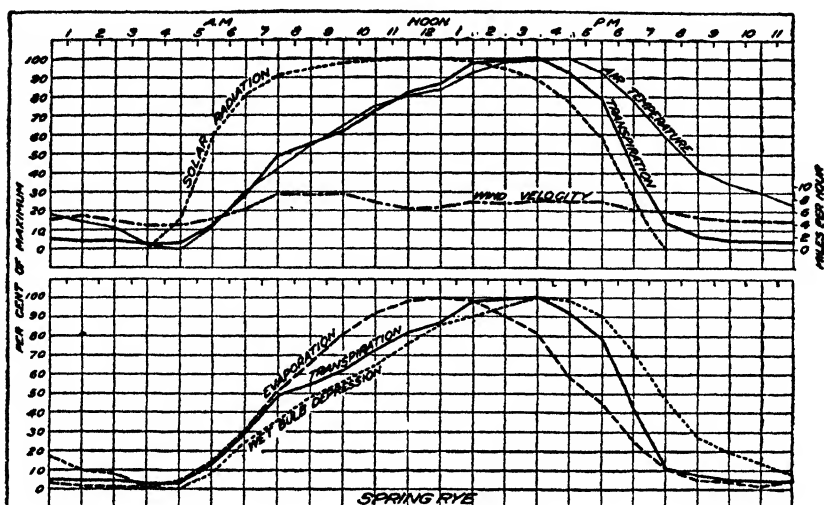


FIG. 12.—Graphs showing hourly transpiration of spring rye and the hourly values of the cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

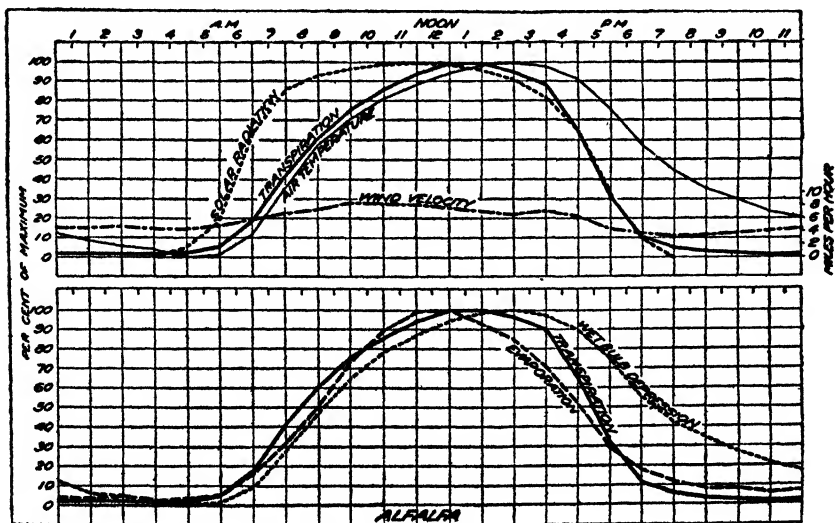


FIG. 13.—Graphs showing the hourly transpiration of alfalfa and the hourly values of cyclic environmental factors, all plotted in percentage of the maximum or maximum range.

This is clearly shown in figure 16, in which the two graphs are plotted for each plant.

The transpiration rises in advance of the temperature in the case of wheat, oats, and alfalfa; approximately with the temperature for rye



It will be seen from these figures that the integrated transpiration for wheat and oats slightly exceeds the integrated radiation and that the reverse is true for rye, sorghum, amaranthus, and alfalfa. The transpiration curves for sorghum, amaranthus, and alfalfa lie almost wholly within the radiation curve. The ratio of the transpiration area to the radiation area is also low in the case of spring rye, owing to the comparatively low rate of transpiration during the morning hours.

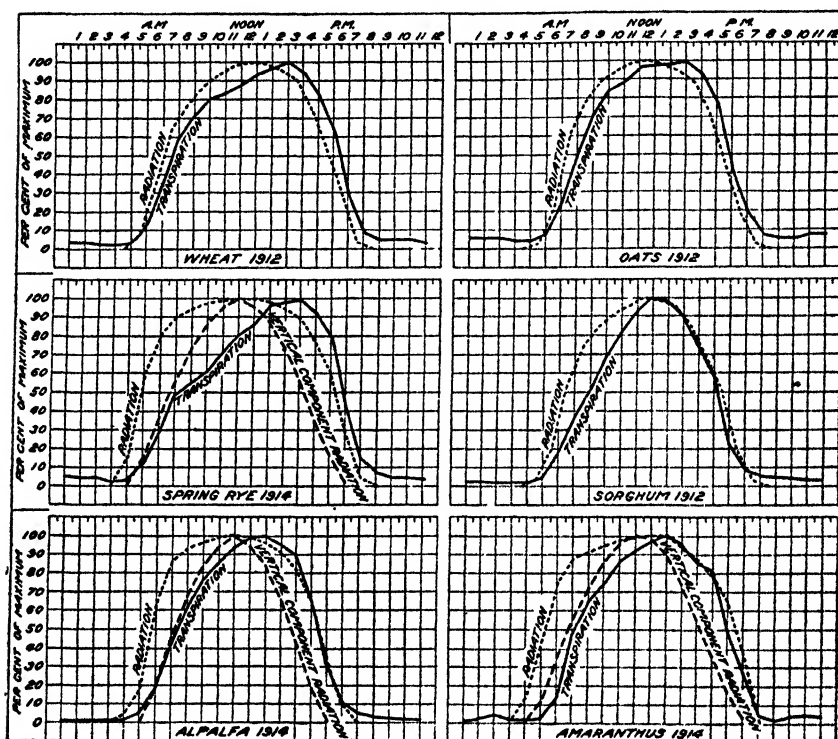


FIG. 16.—Comparison of the form of transpiration graphs with the graphs representing the total radiation and the vertical component of the radiation.

TABLE XXXVI.—A comparison of radiation and transpiration based on the area enclosed by the graphs in figure 17

Plant.	Area bounded by—		Ratio of transpiration to radiation area.	Transpiration.					
	Radiation graph.	Transpiration graph.		Area for day-light hours.	Day-light.	Night.	A. M.	P. M.	11 a. m. to 3 p. m.
					Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Wheat.....	302	310	1.03	298	96	4	44	56	37
Oats.....	289	303	1.05	286	94	6	44	56	39
Rye.....	357	306	.86	290	95	5	38	62	36
Sorghum.....	283	253	.89	240	95	5	43	57	45
Amaranthus.....	346	284	.82	275	97	3	42	58	40
Alfalfa.....	315	271	.86	264	97	3	44	56	43

The last portion of Table XXXVI gives the relative transpiration for different parts of the day. The percentage of the transpiration taking place during daylight is very uniform, ranging from 94 per cent for oats to 97 per cent for amaranthus and alfalfa. The transpiration during the night is remarkably low, ranging from 3 per cent for amaranthus and alfalfa to 6 per cent for oats. The data as presented represent the integration of the transpiration and radiation for hourly intervals, so that the transpiration for the hour interval during which sunrise (or sunset) occurred has been included as daylight transpiration. The ratio can be more accurately determined from the automatic records, which show an

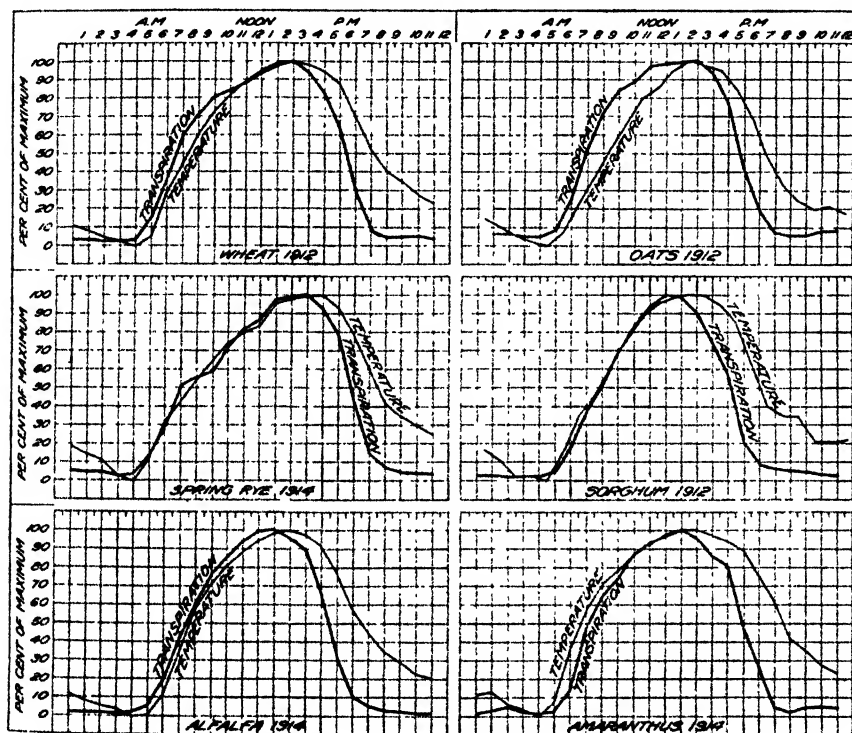


FIG. 17.—Comparison of the transpiration graphs plotted in percentage of the maximum with the temperature graphs plotted in percentage of the maximum range.

average night transpiration less than 5 per cent of that occurring during daylight. This low night transpiration is significant when we consider that the temperature and the saturation deficit are relatively high during the early hours of the night and that the dew point is seldom reached at Akron. The wind velocity at night is also at least one-half the average daylight velocity.

It will be seen from Table XXXVI that the transpiration in the forenoon is lower than in the afternoon, the difference being greatest in the case of rye and least in the case of wheat, oats, and alfalfa. For the

group of plants as a whole, 43 per cent of the transpiration took place before noon and 57 per cent in the afternoon, while the average radiation during the period was slightly greater in the forenoon.

In the last column of the table is given the percentage of transpiration taking place between 11 a. m. and 3 p. m. While these figures are not directly comparable, owing to the difference in the length of the day—i. e., in the number of daylight hours—it is clear that from one-third to

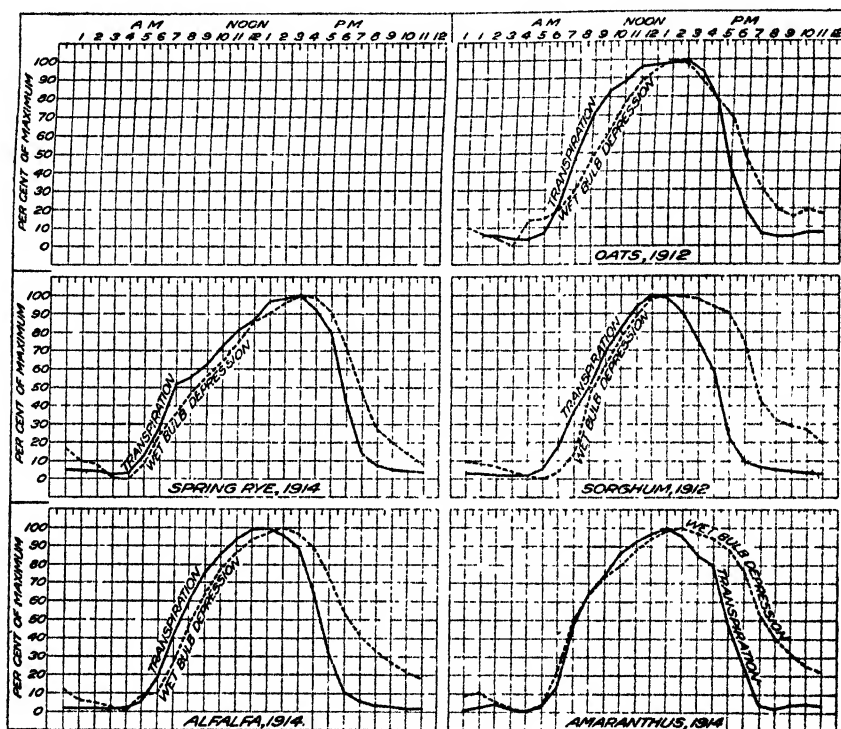


FIG. 18.—Comparison of transpiration with wet-bulb depression, both plotted in percentage of the maximum range.

one-half of the transpiration during the 24-hour period takes place from 11 a. m. to 3 p. m.

#### RATIO OF TRANSPIRATION TO EVAPORATION

Transpiration is often regarded as evaporation modified to some extent by plant structures and plant functions. Both are influenced by radiation, temperature, saturation deficit, and wind. Because of the similarity of the two processes, the evaporation rate has often been used as a standard to which the transpiration is referred.

Livingston (1906 and 1913) has given special attention to the relation of transpiration to evaporation, and has applied the terms "relative

'transpiration,' "transpiring power" (Livingston and Hawkins, 1915), to the ratio of the transpiration rate to the evaporation rate of his porous-cup atmometer. It has been shown<sup>1</sup> that the graphs representing transpiration and the evaporation from the porous-cup atmometer are similar in form, but that their maxima do not as a rule occur at the same time in plants exposed to extreme conditions. Furthermore, when the

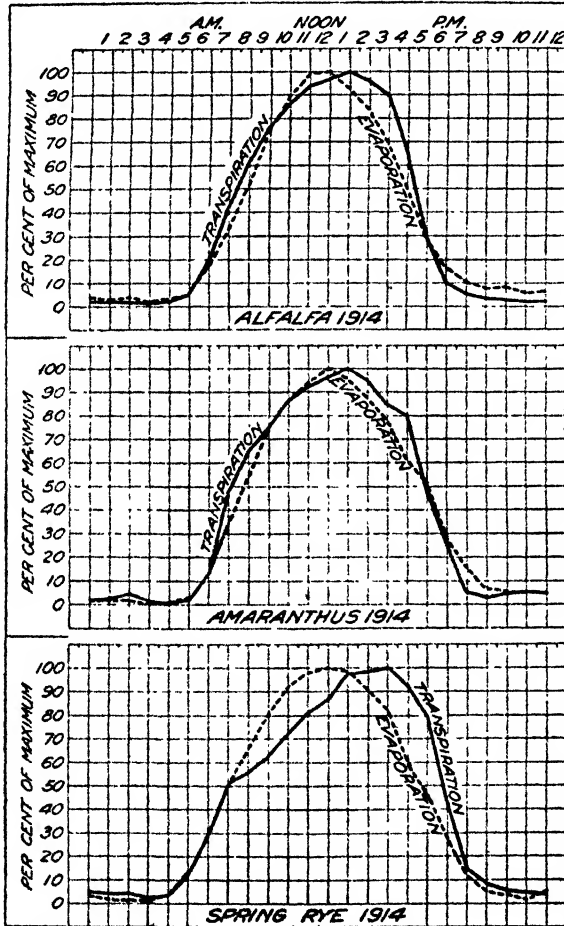


FIG. 19.—Comparison of the transpiration with the evaporation from a free-water surface in a shallow, blackened tank, both plotted in percentage of the maximum range.

ratio of the transpiration to evaporation (the relative transpiration) is plotted against time, the daily graph usually shows two maxima, one in the morning and a second in the afternoon.

Graphs representing the ratio of the transpiration rate of rye, alfalfa, and amaranthus to the evaporation rate are given in figure 20 and show

<sup>1</sup> See also Shreve, 1914; Bakke, 1914.

the maxima referred to in the investigations cited. One maximum occurs in the morning about 7 or 8 o'clock, and a second and greater maximum is found in the afternoon between 4 and 6 p. m.<sup>1</sup> In other words, the transpiration graph shows a tendency to rise earlier in the morning and fall later in the afternoon than the evaporation graph. This is evident in each of the three graphs presented in figure 20.

This result is capable of two quite dissimilar interpretations. If the assumption is made that evaporation constitutes a correct summation of the influence of environment on transpiration, it follows logically that the departure of the transpiration-evaporation ratio from a constant value is due to a decrease or increase in the transpiration coefficient. It must,

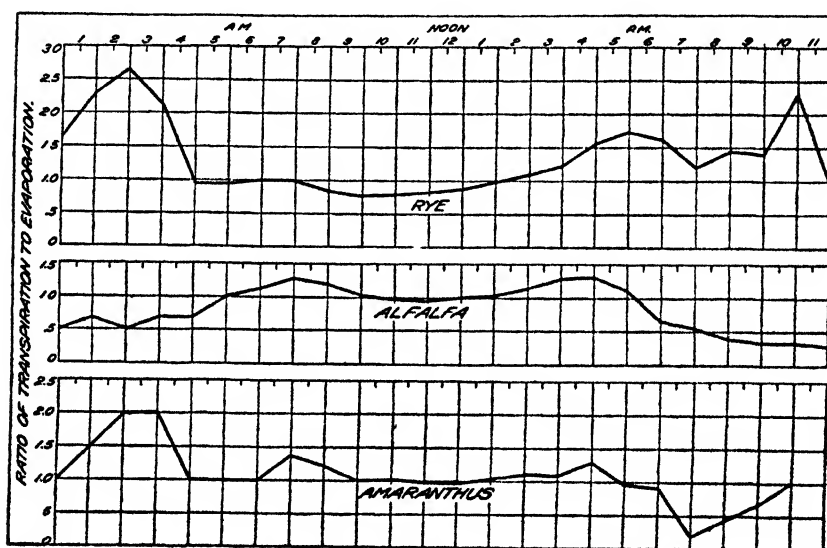


FIG. 20.—Graphs showing hourly ratio of transpiration to evaporation as plotted in figure 19.

however, be recognized that all evaporimeters do not respond to their environment in the same way. A large deep tank does not have the same daily graph as a shallow tank. A filter-paper evaporimeter does not follow the graph of the porous-cup atmometer. If none of these agree, can it be said without further proof that the evaporation rate of any one of them is proportional to the transpiration rate of a plant which responds *freely* to its environment? The fact that the transpiration graph is so uniformly asymmetrical with respect to noon in our determinations and that the evaporation graph is so uniformly symmetrical would indicate that the two processes were not controlled in the same way by the physical factors of the environment. The writers are inclined to the belief that

<sup>1</sup> The hourly values of transpiration and evaporation at night are so small that the observational errors make the ratio uncertain, and the night ratios will consequently not be considered at this time.

the departure of the transpiration from evaporation should not be taken as proof of a change in the transpiration coefficient of the plant and that it is safer for the present not to base conclusions on this assumption but instead to consider directly the factors which influence both transpiration and evaporation.

#### CORRELATION BETWEEN TRANSPIRATION AND ENVIRONMENTAL FACTORS

Two methods have been employed by the writers in making a quantitative investigation of the relationships existing between the transpiration of the plant and the intensity of its environment: (1) The coefficient of correlation between the transpiration and a given environmental factor has been computed as a basis for the determination of the relative influence of the various environmental factors and (2) the relationship between the mean hourly transpiration and the hourly values of the several environmental factors has been computed by the method of least squares, and the relative weights of the different environmental factors determined from the coefficients of the resulting equation. Such a reduction of the data appears highly desirable, for it affords a means of comparison independent of the personal element. The results of the correlation reductions will first be considered.

In computing the correlation coefficients,<sup>1</sup> the individual hourly observations as presented in Tables I to XXVI were used. The data in each instance embrace not less than three days' observations with the transpiration measurements in duplicate, so that the number of pairs of terms—i. e., the "population" considered—approximated 144 for the 3-day periods in the transpiration correlations and in other cases exceeded this number.

The correlation coefficients of the transpiration rate of alfalfa, amaranthus, and rye, with the intensity of the several environmental factors, are presented in Table XXXVII, together with the probable error of the correlation coefficient in each case.

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<sup>1</sup> For a presentation of the theory and the method of computing correlation coefficients, see Yule (1912) and Davenport (1907).

TABLE XXXVII.—Correlation between transpiration and environmental factors

Plant, period, and components.	Correlation coefficient.	Plant, period, and components.	Correlation coefficient.
Alfalfa (long period, Sept. 10 to Oct. 20, 1914):		Alfalfa (June 18, 19, 21, 1914)—Continued.	
Radiation and transpiration.....	0.840±0.009	Wind velocity and transpiration.....	0.626±0.036
Temperature and transpiration.....	.819±.011	Wind velocity and radiation.....	.641±.046
Wet-bulb and transpiration.....	.822±.011	Vertical radiation and transpiration..	.818±.013
Evaporation and transpiration.....	.838±.011	Amaranthus (July 7 to 9, 1914):	
Wind velocity and transpiration.....	.485±.026	Radiation and transpiration.....	.844±.016
Wind velocity and radiation.....	.302±.030	Temperature and transpiration.....	.849±.016
Alfalfa (Oct. 16 to 20, 1914):		Wet-bulb and transpiration.....	.842±.016
Radiation and transpiration.....	.886±.010	Evaporation and transpiration.....	.946±.006
Temperature and transpiration.....	.859±.012	Wind velocity and transpiration.....	.683±.031
Wet-bulb and transpiration.....	.843±.013	Wind velocity and radiation.....	.776±.032
Evaporation and transpiration.....	.929±.006	Vertical radiation and transpiration...	.863±.014
Wind velocity and transpiration.....	.353±.039	Rye (June 22 to July 3, 1914):	
Wind velocity and radiation.....	.275±.084	Radiation and transpiration.....	.820±.014
Vertical radiation and transpiration..	.862±.011	Temperature and transpiration.....	.854±.011
Alfalfa (June 18, 19, 21, 1914):		Wet-bulb and transpiration.....	.748±.018
Radiation and transpiration.....	.861±.015	Evaporation and transpiration.....	.894±.033
Temperature and transpiration.....	.788±.021	Wind velocity and transpiration.....	.376±.036
Wet-bulb and transpiration.....	.852±.008	Wind velocity and radiation.....	.353±.047
Evaporation and transpiration.....	.888±.012	Vertical radiation and transpiration..	.766±.017

An inspection of the correlation table will show that, excluding evaporation, the highest correlation is obtained between radiation and transpiration. The correlation coefficient for these components is remarkably uniform for the different crops and periods, ranging from 0.82 to 0.88, the lowest value occurring in the case of rye, as one might expect from the form of the transpiration graph.

The similarity in the form of the composite graphs for air temperature and wet-bulb depression would lead to the expectation that their correlation coefficients with transpiration would be similar and the coefficients are in fact nearly the same. The only exceptions are (1) alfalfa (June period), in which the wet-bulb depression shows the higher correlation with transpiration; and (2) rye, in which temperature is the more closely

correlated. Reference to figures 12 and 17 shows the unusually close agreement between the composite transpiration graph for rye and the temperature graph.

The correlation coefficient of temperature (or wet-bulb depression) and transpiration also agrees approximately with that of radiation and transpiration. In other words, it appears from a consideration of these coefficients that radiation, temperature, and wet-bulb depression show an equally close association with the daily transpiration cycle. The correlation of temperature and wet-bulb depression with transpiration may, however, be looked upon as being in part associative with radiation rather than causative, as will appear from the following considerations.

The degree of correlation<sup>1</sup> between radiation and transpiration (from 0.82 to 0.88) indicates that the radiation determines the transpiration to the extent of from 0.67 to 0.77, the square of the correlation coefficients, if radiation is regarded as the primary causative factor. The remainder (0.33 to 0.23) is to be ascribed to other factors. If temperature is taken as a causative factor of transpiration, the correlation coefficients show a dependence of transpiration upon temperature of from 0.62 to 0.74; but this is far in excess of the remainder (0.33 to 0.23) to be accounted for. In other words, the sum of the squares of the two correlation coefficients is in excess of unity. This means, then, that temperature and radiation are intercorrelated. A similar intercorrelation exists between radiation and wet-bulb depression, and an exact differentiation is impossible. However, since these factors are physically dependent upon radiation, we may assign to radiation the total effect indicated by the correlation coefficient, keeping always clearly in mind the assumption involved. On this basis the radiation intensity determines two-thirds to three-fourths of the transpiration at Akron on clear days; or all other factors combined have only from one-third to one-half the influence of radiation.

On the other hand, if it is preferred to look upon radiation, temperature, and wet-bulb depression as direct independent causative factors (which must also be recognized as involving a specific assumption to this effect), then it is evident from Table XXXVII that these factors play approximately an equal part in determining transpiration on clear days. Not only are the correlation coefficients very nearly the same for the different factors with a given crop, but they vary but slightly for the different plants investigated.

<sup>1</sup> While a correlation coefficient of unity denotes perfect correlation, a correlation coefficient of less than unity must not be interpreted as determining the relationship in proportion to the magnitude of the correlation coefficient, for even in the case of a primary causative factor the relationship can not be greater than the square of the correlation coefficient. For example, a correlation coefficient of 0.707 between a causative and a resultant term indicates a dependence of the latter upon the former of 0.5—i. e., the square of 0.707. This may be easily demonstrated by computing the correlation coefficient between either of two series of numbers, each having a normal frequency distribution, with the product of one series by the other. The correlation coefficient of the product series with either primary series will be found to be 0.707. In other words, each series determines the product series to the extent of 0.5, while the two series together determine the product series absolutely.



In order to decide between these two assumptions, other evidence is necessary; and this may be found in a consideration of the transpiration during the night—i. e., when the radiation received by the plants is nil.

In Table XXXVIII are summarized the transpiration and wet-bulb depression (in percentage of the maximum) and the air temperature (in percentage of the maximum range) for the hours 3 to 4 a. m. and 8 to 9 p. m. It is evident from the table that a simultaneous diminution in the wet-bulb depression of one-fourth of its maximum and in temperature of one-third of its maximum range results in a drop of only 3 per cent in the transpiration rate. This would seem to indicate that the high correlation obtained between transpiration and air temperature (or wet-bulb depression) is largely due to the direct correlation between radiation and temperature (or wet-bulb depression).<sup>1</sup>

TABLE XXXVIII.—*Comparison of transpiration, temperature, and wet-bulb depression at 3 to 4 a. m. and 8 to 9 p. m.*

Crop or period.	Per cent of maximum transpiration.			Per cent of maximum temperature.			Per cent of maximum wet-bulb depression.		
	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.	3 to 4 a. m.	8 to 9 p. m.	Difference in a. m. and p. m. reading.
Wheat.....	3	5	2	3	40	37	.....	.....	.....
Oats.....	4	6	2	2	24	22	21	37	16
Rye.....	3	7	4	3	42	39	17	39	22
Sorghum.....	2	5	3	2	34	32	25	48	23
Amaranthus.....	1	3	2	4	42	38	12	45	33
Alfalfa.....	2	3	1	4	35	31	15	42	27
June period.....	2	3	1	3	49	46	19	47	28
October period.....	1	7	6	5	32	27	2	38	36
Mean.....	.....	.....	3	.....	.....	34	.....	.....	26

If we ascribe to radiation a causative effect equal to that indicated by the correlation coefficient with transpiration, it becomes possible also to investigate the influence of wind velocity on transpiration by a process of elimination similar to that employed above.

Transpiration in still air is somewhat less than in moving air, since the latter tends to reduce the distance that the transpired moisture must move in order to find free-air conditions. In other words, the wind tends to increase the diffusion gradient, and so increases the transpiration (or evaporation) rate. But a slight movement appears to satisfy this condition, and the correlation coefficients between wind and transpiration (Table XXXVII) show that the variation in wind at Akron, where some wind nearly always occurs, has little influence on the trans-

<sup>1</sup> In opposition to this view it may be argued that the plants from 3 to 4 a. m. are more turgid than from 8 to 9 p. m. This is undoubtedly true, but it is also true that during the last named period the plants are more turgid than at 2 or 3 p. m., the period during which the maximum transpiration rate was observed.

piration rate. In arriving at this conclusion it is again necessary to consider the correlation not only between wind and transpiration, but also between wind and radiation. If the wind influences transpiration independently of its association with radiation, the wind velocity must show a higher correlation with transpiration than with radiation. This occurs only during the long alfalfa period, in which there appears to be a slight effect due to wind. In all other cases the wind correlation with transpiration differs from the wind correlation with radiation by an amount not greater than the probable error of the difference. Here, again, we are making the specific assumption that the radiation is the primary causative factor, so that if wind is associated with transpiration to an extent no greater than with radiation its effect on transpiration is slight. This assumption is here again supported by the fact that the transpiration is extremely low during the night hours, although the wind is blowing.

If transpiration and evaporation are largely determined by the same factors or, in other words, if transpiration is essentially a physical process, then a high correlation between transpiration and evaporation is to be expected. Reference to Table XXXVII will show that the correlation of evaporation with transpiration ranges from 0.84 to 0.95. The latter value is slightly higher than the maximum correlation (0.89) of radiation with transpiration and shows that 0.9 of the transpiration was in this instance determined by the same factors which determined the transpiration.

The relation of evaporation to transpiration is to be considered as associative rather than causative, both responding to the same environmental factors, but not necessarily in precisely the same way or to the same degree. The extent of this association furthermore depends upon the manner in which evaporation is measured. For example, the evaporation rate from a free-water surface in a very shallow tank conforms much more closely to the transpiration rate than when a deep tank is used, since the latter, on account of its large heat capacity, stores up a large amount of energy which is dissipated through evaporation during the night. It is evident that the evaporimeter must simulate the plant system as nearly as possible in absorption and heat capacity if a high degree of correlation between the two is to be attained.

#### LEAST-SQUARE RELATIONSHIPS BETWEEN TRANSPIRATION (OR EVAPORATION) AND ENVIRONMENTAL FACTORS

The method of least squares affords a means of determining the relative influence of the various environmental factors upon the transpiration. In these least-square reductions (Merriman, 1893, and Bartlett, 1915) the mean hourly values have been used, and it has been assumed that the relationship is linear in character—i. e., that the transpiration varies directly in proportion to the intensity of the environmental factors.

The results of the least-square reductions are presented graphically in figures 21 and 22. In all cases, the vertical component of the radiation has been employed rather than the radiation on a surface normal to the sun's rays. The reason for this will be apparent from an inspection of the radiation and transpiration charts, where it will be seen that during the early morning hours the slope of the radiation graph is much greater than that of the transpiration graph for rye, alfalfa, and amaranthus. In other words, the transpiration rate does not increase nearly as rapidly as the normal component of the radiation during the early daylight hours. In a field of grain or alfalfa, considered as a whole, it is evident that the vertical component of the radiation would alone be effective. In the case of an isolated pot of plants standing on the transpiration scale, the horizontal component would also be effective. The extent to which this enters can not be directly determined, however, and in the following discussion the vertical component has been used throughout.<sup>1</sup>

#### TRANSPIRATION AS DETERMINED BY RADIATION AND TEMPERATURE

The observed and computed transpiration graphs, the latter based on the assumption that the vertical component of the radiation and the air temperature are the primary controlling factors in transpiration, are given in figure 21. The computed equations are as follows:

For rye.....0.384  $R_v + 0.642 \theta = T$ ;

For alfalfa.....0.514  $R_v + 0.539 \theta = T$ ;

For amaranthus.....0.546  $R_v + 0.443 \theta = T$ ;

in which

$R_v$  is the vertical component of radiation,

$\theta$  is the temperature rise, and

$T$  is the transpiration.

In the above equations and in those which follow the hourly values for each term are expressed as a percentage of the maximum. In other words, the general dimensionless equation is of the form:

$$a \frac{R'_v}{R'_{v_{max}}} + b \frac{\theta' - \theta'_0}{\theta'_{max} - \theta'_0} = \frac{T'}{T'_{max}}$$

in which the primed quantities represent observed values.

<sup>1</sup> Calculation of the vertical component of radiation.—If  $R$  represents the normal component of the radiation of the sun,  $R_v$  the vertical component, and  $h$  the altitude—i. e., the angular distance of the sun above the horizon—then:  $R_v = R \sin h$ .

Expressing the altitude in terms of declination and hour angle (Smithsonian Institution, 1894, p. 1xviii), we have  $\sin h = \sin \phi \sin \delta + \cos \phi \cos \delta \cos t$ , in which

$\phi$ —the latitude of the place of observation;

$\delta$ —the declination of the sun—i. e., the angular distance above or below the Equator (from U. S. Navy Dept., 1912); and

$t$ —the hour angle—i. e., the angle between the meridian plane through the place and the meridian plane through the sun.

Substituting, we have:

$R_v = R (\sin \phi \sin \delta + \cos \phi \cos \delta \cos t)$ .

The daily observations are expressed on the basis of mean sun time, which introduces a slight error in the calculation of the vertical radiation component.

An inspection of the curves in figure 21 will show that the computed graph agrees with the observed transpiration graph much better in the morning than in the afternoon.<sup>1</sup> The computed graph always reaches its maximum in advance of the observed graph. The greater departures occur during the early afternoon and early evening. The agreement is by no means as good as is to be desired, and the graphs show clearly that transpiration can not be completely accounted for on the assumption

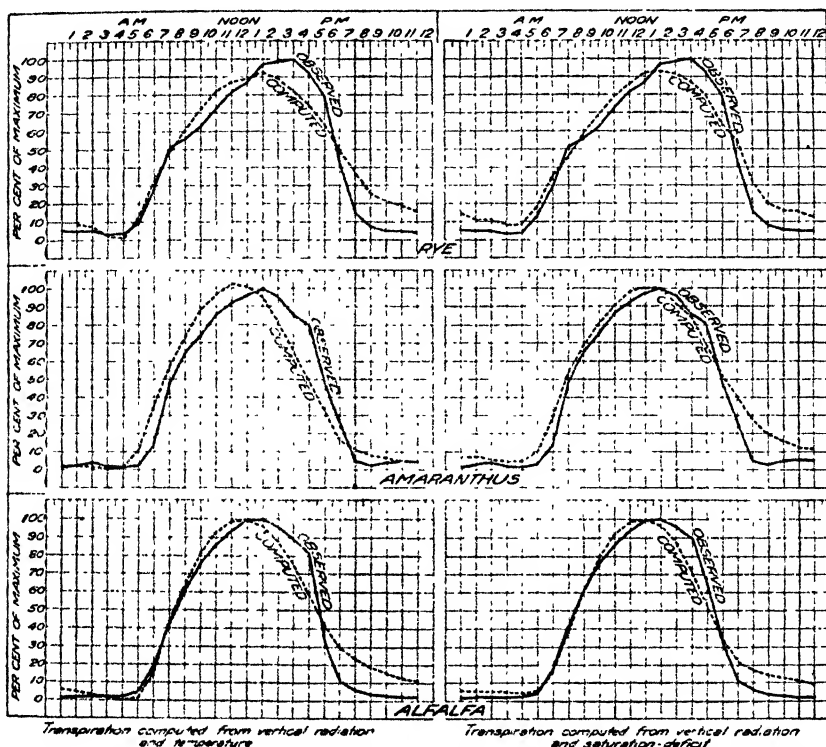


FIG. 21.—Graphs showing the observed transpiration with that computed from vertical radiation and temperature (on the left) and from vertical radiation and saturation deficit (on the right).

that the vertical component of radiation and the rise in temperature are the controlling factors.

The relative values of the computed coefficients are of interest. In the case of alfalfa, the radiation is weighted 0.97 relative to temperature; amaranthus, 1.23; and rye, 0.60. In this connection it should be recalled that rye shows a sudden change in the slope of the transpiration graph in the morning, differing markedly from alfalfa and amaranthus in this respect.

<sup>1</sup>Since preparing figures 21 and 22 a recalculation based on more exact determinations of the vertical component of radiation has given computed values of transpiration and evaporation which are in somewhat closer agreement with the observed values during the daylight hours than those indicated in the charts. The coefficients in the equations are based upon the revised calculation.

## TRANSPIRATION AS DETERMINED BY RADIATION AND SATURATION DEFICIT

Corresponding graphs based upon vertical radiation and saturation deficit are also given in figure 21. The values for the latter term are computed from the mean hourly wet-bulb depression and the corresponding hourly air temperatures. The resulting equations follow:

For rye .....  $0.455 R_v + 0.622 D = T$ ;

For alfalfa.....  $0.538 R_v + 0.553 D = T$ ;

For amaranthus.....  $0.538 R_v + 0.481 D = T$ ;

in which  $D$  represents the saturation deficit expressed as a percentage of

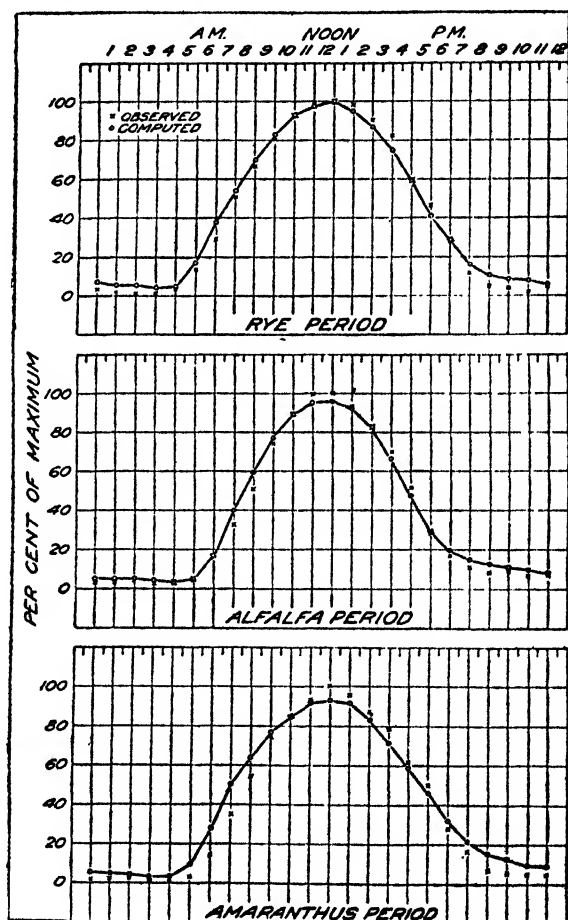


FIG. 22.—Graphs showing the observed evaporation with that computed by least-square methods from the vertical component of radiation and the saturation deficit.

the maximum, and the other symbols have the same meaning as before. An inspection of the graphs shows them to be similar in form to those computed from radiation and temperature. The coefficients are also

similar to those of the former series. The ratio of the radiation to the saturation-deficit coefficient for the different plants is as follows: Rye, 0.73; alfalfa, 0.97; amaranthus, 1.12. The equation for rye again shows the radiation to have the lesser influence of the two factors considered, while in the case of the other two plants, the radiation has an equal or greater influence. The equations for the latter plants are in fair agreement, but in all cases discrepancies occur between the observed and computed curves, particularly during the early afternoon and early evening hours.

#### EVAPORATION AS DETERMINED BY RADIATION AND SATURATION DEFICIT

The evaporation rate from the shallow, blackened tank for the three transpiration periods just considered has also been computed, assuming the vertical radiation and the saturation deficit to be the controlling environmental factors. The observed and computed evaporation graphs are given in figure 22. The agreement during the rye and alfalfa periods is very satisfactory, but during the amaranthus period the departures are greater. The evaporation equations for the several periods are as follows:

Rye period . . . . .  $0.787 R_r + 0.292 D = E$ ;

Alfalfa period . . . . .  $0.680 R_v + 0.360 D = E$ ;

Amaranthus period . . . . .  $0.563 R_r + 0.411 D = E$ ;

in which  $E$  represents the evaporation expressed as a percentage of the maximum.

It will be observed that the radiation has a preponderating influence in each instance.

#### DISCUSSION OF LEAST-SQUARE REDUCTIONS

The least-square reductions again emphasize the fact that the transpiration response to changing environmental conditions is not the same for different plants. In other words, the distribution of the transpiration loss through the day varies with different plants. Furthermore, the distribution of the transpiration loss differs from the distribution of the evaporation loss from a shallow tank. As a whole, the agreement between observed and computed evaporation is much closer than between observed and computed transpiration. Either some factor operative in transpiration yet remains to be accounted for or the transpiration system changes its coefficient during the day. The latter condition may result from stomatal control or through the inability of the plant to secure sufficient water to maintain complete turgidity during the day. The fact that the evaporation on clear days can be satisfactorily accounted for from a consideration of radiation and saturation deficit indicates that the essential environmental factors have been considered and suggests that the outstanding differences between observed and computed trans-

piration are due to differences in the plants or to some change in the plant as the day progresses.

It is probable that plants differ also in their response to solar energy, the absorption coefficient of different plants not being the same, while the dissipation of the energy absorbed is quite different in different plants. In other words, the ratio of the energy dissipated through transpiration and lost by the plant through emissivity is not the same for all species. Such changes probably occur also in the same plant during the daily cycle, which would modify the transpiration coefficient irrespective of the changes in physical conditions.

#### SUMMARY

This paper deals with measurements of transpiration on clear days at Akron, Colo., in relation to environmental factors. The plants, which included wheat, oats, rye, sorghum, alfalfa, and amaranthus, were grown in large sealed pots of the type used in water-requirement measurements, containing sufficient soil (about 115 kgm.) to enable the plants to make a normal growth. The transpiration was determined by weighing, four automatic platform scales recording each 20-gm. loss being used for the purpose. Automatic records were simultaneously made of the radiation intensity, the air temperature, the depression of the wet-bulb thermometer, the evaporation, and the wind velocity. The radiation intensity and the wet-bulb depression were measured by differential telethermographs, and the evaporation rate from a free-water surface was determined by mounting a shallow, blackened evaporation tank 3 feet in diameter on an automatic platform scale.

Composite graphs are presented, showing the mean hourly transpiration rate for each of the plants considered, together with the mean hourly values of the radiation, air temperature, wet-bulb depression, and wind velocity for the transpiration period and also the mean hourly evaporation rate. On the basis of the form of the curves the transpiration graphs may be grouped into two classes having characteristic features. The cereals show a marked change in the slope of the transpiration graph in the forenoon unaccompanied by corresponding changes in the environmental factors. On the other hand, the forage plants and amaranthus give little or no indication of such a change. This flattening of the graphs in the case of the cereals appears to be due to some change in the plant, resulting in a reduction in the transpiration rate below what would be expected from the form of the curve during the early morning hours.

The hourly transpiration rate of the cereals on clear days increased steadily, though not uniformly, from sunrise to a maximum value, usually reached between 2 and 4 p. m., after which it fell rapidly to the night level. The transpiration graphs for sorghum, alfalfa, and amaranthus were somewhat more symmetrical with respect to midday, reaching

their maximum between noon and 2 p. m., after which they fell approximately with the radiation.

The transpiration during the night at Akron is very low, being only 3 to 5 per cent of the transpiration during the daylight hours.

The radiation graphs are practically symmetrical with respect to noon, showing that the days selected were relatively clear. When all the mean hourly values are expressed as a percentage of the maximum, the radiation intensity rises in advance of the transpiration (and in advance of all the other environmental factors as well) and falls either in advance of the transpiration or with it, depending on the plant considered. Radiation then may be looked upon as the primary causative factor in the cyclic changes.

The air temperature and wet-bulb depression graphs are very similar in form, since the latter can be determined from the former on days in which the absolute humidity of the air is not changing. The transpiration graphs usually rise and always fall in advance of air temperature.

The evaporation graph from the shallow, blackened tank (water approximately 1 cm. in depth) is similar in form to the graph representing the vertical component of radiation. This is to be expected, since only the vertical component would strike the horizontal water surface. The evaporation graph rises and falls with, or slightly later than, the vertical component of radiation.

Computation of the correlation coefficients between transpiration and the various environmental factors shows the radiation, air-temperature, and wet-bulb depression to be correlated with transpiration approximately to the same degree. The correlation coefficients of transpiration with radiation range from 0.82 to 0.89; with temperature from 0.77 to 0.86; and with wet-bulb depression, from 0.75 to 0.85. These figures show the intercorrelations existing among the environmental factors, since the sum of the squares of the coefficients of independent causative factors influencing transpiration can not exceed unity. If radiation is taken as the primary causative factor, the correlation coefficients show that 0.67 to 0.77 of the transpiration on clear days under Akron conditions is determined by the radiation intensity.

If the environmental factors are considered as independent, their relative influence on transpiration may be determined by the method of least squares. In the case of alfalfa and amaranthus, the vertical component of radiation and the temperature enter into the determination of transpiration in the ratio of 1 to 1, approximately; and the corresponding ratios for vertical radiation and saturation deficit are approximately the same. On the other hand, in the case of rye, the radiation by this method of reduction shows less influence than either temperature or saturation deficit on the transpiration rate, which from 9 a. m. to 2 p. m. shows a marked departure from the graph indicated by the transpiration during the early morning hours.



Least-square reductions of the dependence of transpiration upon radiation and air temperature or upon radiation and saturation deficit do not account entirely for the observed transpiration, although a satisfactory agreement between computed and observed evaporation is obtained by the use of these environmental factors. This indicates that the plant undergoes changes during the day which modify its transpiration coefficient. In other words, our results support the conclusion of other investigators that plants under conditions favoring high evaporation do not respond wholly as free evaporating systems, even if bountifully supplied with water and no visible wilting occurs.

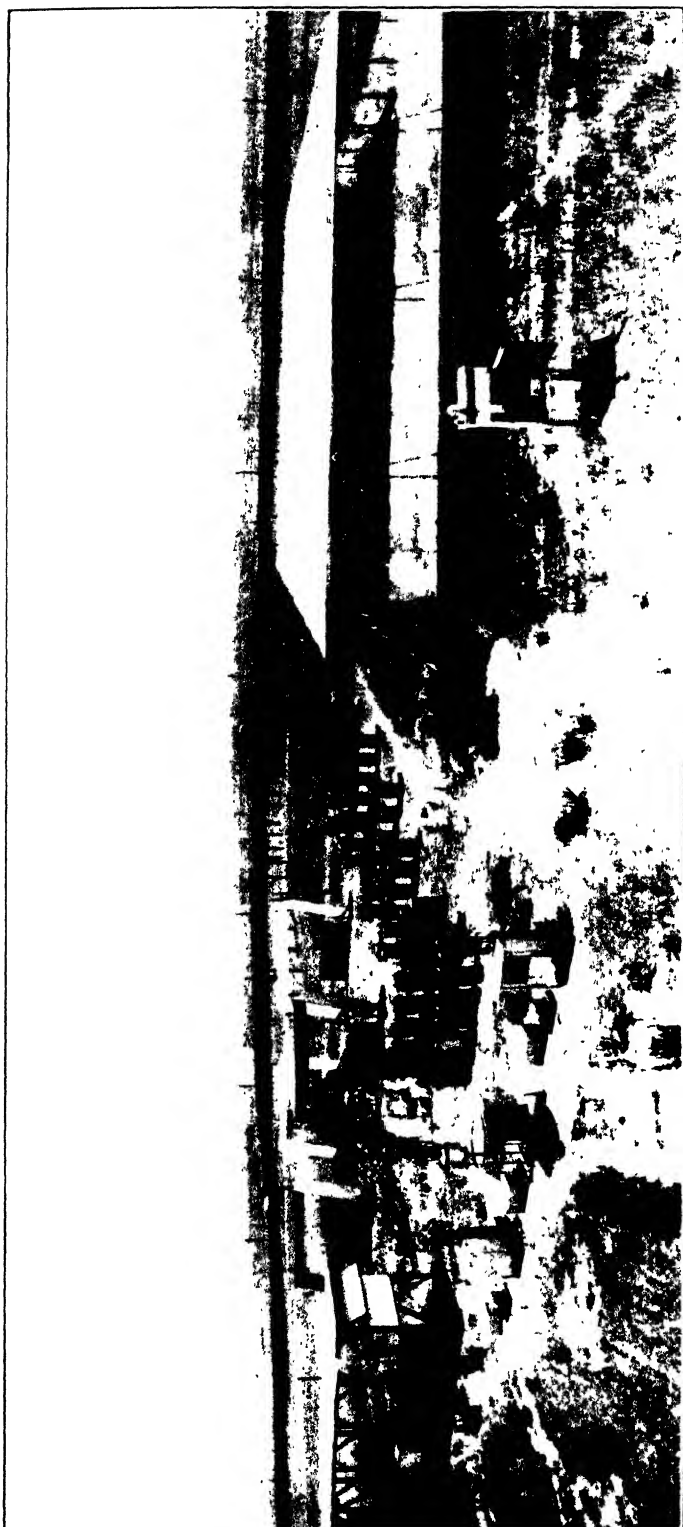
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### PLATE LIII

General view of the water requirement and transpiration experiments at Akron, Colo., on July 8, 1913. The large, screened inclosure in which the transpiration measurements were made in 1912 is shown at the right. The small instrument shelter in the foreground contained differential thermographs for measuring wet-bulb depression and solar radiation. The glass envelope surrounding the bulb of the radiation instrument may be seen on the top of the instrument shelter. At the left in the foreground is shown balance A, the front of the box open, and the recording device uncovered at the left. This balance is carrying a pot of sunflower. The next balance, B, carries the evaporation tank; balance C, another sunflower pot; and balance D, under the shade at the left, a third sunflower pot. The exposure of balances A and B, as used in the 1913 and 1914 determinations, is shown in this illustration.





#### PLATE LIV

Fig. 1.—Wheat on automatic balances in the screened inclosure, July 3, 1912, showing the exposure and arrangement of the 1912 experiments.

Fig. 2.—Automatic balances A, B, and C; A and C carry pots of cowpea and B carries the evaporation tank. This shows the exposure of the plants in the 1913 and 1914 transpiration experiments.

#### PLATE LV

Fig. 1.—A pot of alfalfa showing the growth and size of plants used in the transpiration experiments. The pot is 26 inches high and 16 inches in diameter.

Fig. 2.—A pot of *Amaranthus retroflexus* of the type used in the transpiration measurements.

Fig. 3.—Evaporation tank mounted on automatic balance. The reservoir is shown above in the back. The tank has an area of 6,540 sq. cm. and the water is maintained at a depth of 1 cm. The balance recorder is shown at the right and the anemometer at the left in the background.







# EFFECT OF NATURAL LOW TEMPERATURE ON CERTAIN FUNGI AND BACTERIA

By H. E. BARTRAM,

Assistant Plant Pathologist, Vermont Agricultural Experiment Station

The effect of the very intense cold of northern winters on the life and viability of fungi and bacteria does not seem to have been tested extensively, yet its importance in checking the spread of plant infections from these sources would appear to be very great.

Wolf<sup>1</sup> has shown that certain parasitic and saprophytic fungi remain present and alive in Nebraska orchards during autumn, winter, and spring. The majority of the species are saprophytic, the more common ones being *Alternaria* spp., *Cladosporium* spp., and *Penicillium expansum*. Only one parasite, the cause of leafspot, was present in abundance regardless of temperature. He found more spores in the air in neglected orchards than in well-cared-for ones and also found them to be much more abundant everywhere than commonly has been supposed. All his determinations were made by exposing plates at various places in the orchard and then carefully studying and determining the colonies after they had developed.

In the present study certain known fungi and bacteria were exposed in pure cultures to the low temperature of the winter months. The organisms were started upon nutrient agar in test tubes—i. e., allowed to grow at laboratory temperature for about one week after inoculation—and then these cultures were placed in a corncrib where there was a free circulation of air, but where they were protected from the rain and snow.

The tubes were inoculated between December 10 and 16 and were exposed in the outhouse on December 21, with the exception of *Actinomyces organicus*, which was not exposed until December 31. The cultures were undisturbed throughout the winter, during which time a minimum temperature of  $-24^{\circ}$  C. was reached. The medium did not dry up to any extent, but was rather moist when brought into the laboratory, as the frequent freezings and thawings seemed to impair the solidifying power of the agar.

On April 14 the cultures were brought into the laboratory and tested immediately for vitality. This was done by transferring part of the exposed culture to fresh nutrient-agar slants and allowing the new inoculations to grow at room temperature. In all cases except one the response to fresh agar was soon evident, but in the case of *Actinomyces*

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<sup>1</sup> Wolf, F. A. The prevalence of certain parasitic and saprophytic fungi in orchards, as determined by plate cultures. *In* Plant World, v. 13, no. 7, p. 164-172, fig. 1; no. 8, p. 190-202, fig. 4-5. 1910.

*chromogenus* the organism was probably killed by the low temperature. A large proportion of the conidia of both strains of *Sclerotinia cinerea* were found to be capable of germination. Table I gives the organisms and materials used and the results obtained.

TABLE I.—Results of tests for vitality of various organisms after exposure to low temperatures (1912-13)

Organism.	Medium.	Response of the mycelium.
<i>Cephalothecium roseum</i> .....	Synthetic agar ..	Excellent growth in 2 days, with production of spores.
<i>Sclerotinia cinerea</i> (Vermont culture) .....	.....do.....	Excellent growth in 36 hours; many conidia produced.
<i>Alternaria solani</i> .....	Lima-bean agar ..	Good growth after 2 days.
<i>Cylindrosporium pomi</i> .....	Synthetic agar ..	Good growth after 6 days; slow to start.
<i>Sphaeropsis malorum</i> .....	.....do.....	Slow growing at first; very good later.
<i>Fusarium</i> sp. of conifers .....	.....do.....	Excellent growth in 5 days over entire slant. Two trials needed to get results.
<i>Glomerella rufomaculans</i> .....	.....do.....	Started after 1 day and grew quickly.
<i>Sclerotinia cinerea</i> (culture from New Jersey Experiment Station) .....	.....do.....	Very good growth in 5 days.
<i>Plowrightia morbosa</i> .....	.....do.....	Excellent growth after 1 day.
<i>Venturia inequalis</i> .....	.....do.....	Good growth in 5 days with fruiting. Two trials necessary to get results.
<i>Actinomyces organicus</i> .....	Plain agar .....	Good growth in 2 tubes in 6 days.
<i>Actinomyces chromogenus</i> .....	.....do.....	No growth after a month. No growth on second trial.

The results secured during the winter of 1912-13 were so encouraging that further trials were made the following winter. Several organisms not tested previously were exposed with those first used, and the varieties used the first winter were tested on different media.

Since organisms in nature would be necessarily in a dry state during the winter and without much, if any, nourishment, it was the aim of the author to imitate for his pure cultures these conditions so far as possible. Accordingly, dry cultures of the various fungi chosen for this work, as well as the cultures on nutrient media, were exposed during the winter of 1913-14. These dry cultures were made by removing the growth of the fungus from the surface of the agar with a sterile needle and placing it in an empty, plugged, sterile test tube. A little of the agar was necessarily carried over with the fungus, but not enough to supply it with moisture or food for any length of time. In the case of the bacteria, some of the material from an agar slant was swabbed out with pieces of sterile cotton and placed in plugged, sterile test tubes. All of the cultures thus transferred were dried for 10 days in a warm closet in the laboratory before being exposed. It was expected that the question of food could be practically eliminated, while moisture was available only as it was carried by the air to the cultures.

The cultures were all prepared earlier the second season, and they were placed in the same corncrib on December 13, 1913. Along with the cultures was placed a Draper self-registering thermometer, in order that a comparative record might be kept of the temperatures to which the organisms would be exposed. This thermometer did not register accurately below  $-27^{\circ}\text{C}.$ , so that during the three periods when the tempera-

ture fell below that point the official records of the Weather Bureau were considered as applicable to this test. The temperature was recorded from the date of exposure to March 1, 1914.

Table II summarizes briefly the extremes of temperature in the corncrib and also gives the lowest official record during each week of exposure.

TABLE II.—*Temperature records at Burlington, Vt., during winter of 1913-14*

Date.	Range in corncrib.	Lowest official record.
	°C.	°C.
Dec. 12-19, 1913.....	7 to -13	-14
Dec. 19-26, 1913.....	4.5 to -9	-9.4
Dec. 26, 1913-Jan. 2, 1914.....	-4.5 to -23	<sup>a</sup> -24.4
Jan. 2-9, 1914.....	0 to -19	-20.5
Jan. 9-16, 1914.....	2 to -29	<sup>b</sup> -32
Jan. 16-23, 1914.....	-4 to -22.8	-23.3
Jan. 23-30, 1914.....	7 to -20.5	-22
Jan. 30-Feb. 6, 1914.....	4.5 to -14	.....
Feb. 6-13, 1914.....	2.8 to -26.6	<sup>c</sup> -30
Feb. 13-20, 1914.....	-4 to -26	<sup>d</sup> -27.7
Feb. 20-28, 1914.....	10 to -23.3	<sup>e</sup> -25

<sup>a</sup> Jan. 1.

<sup>b</sup> Jan. 14.

<sup>c</sup> Feb. 12.

<sup>d</sup> Feb. 16.

<sup>e</sup> Feb. 25.

Tests were made of the vitality of the cultures on January 17, February 21, and March 27. These tests were made by transferring some of the growth from duplicate tubes of all the exposed cultures to fresh media of corresponding kind and holding at room temperature (19 to 22° C.) for several days. An abundance of tubes had been prepared, so that when the transfers showed no growth at the end of seven days two more exposed tubes could be brought in and tested. It will be noted that the first test for vitality was made on January 17, immediately following the extremely cold weather of January 13 and 14, when the official record was -30° and -32° C., respectively. Many of the organisms had withstood temperatures of -24° the previous winter, so it was not thought necessary to test any of them until they had experienced more severe cold. In Table III the results of these tests are summarized. Each sign used indicates the response of one culture; the plus (+) signs indicate growth, and the minus (-) signs mean that the culture was dead; "c" denotes contamination of the culture.

TABLE III.—Results of tests for vitality of various organisms after exposure to low temperatures (1913-14)

Organism.	Medium.	Date.	Result.	Date.	Result.	Date.	Result.
<i>Sclerotinia cinerea</i> .....	Synthetic agar .....	Jan. 17	++	Feb. 21	++	Mar. 27	++
<i>Alternaria solani</i> .....	Dry synthetic agar .....	17	++	21	++	27	++
	Synthetic agar .....	17	—	21	—	27	—
	Dry synthetic agar .....	17	++	21	++	27	++
	Lima-bean agar .....	17	++	21	++	27	++
<i>Cylindrosporium pomi</i> .....	Synthetic agar .....	17	++	24	++	27	++
	Dry synthetic agar .....	17	—	24	—	27	—
<i>Sphaeroptis malarum</i> .....	Synthetic agar .....	17	++	24	++	27	++
	Dry synthetic agar .....	17	—	24	—	27	—
<i>Fusarium</i> sp. of conifers .....	Synthetic agar .....	17	++	24	++	27	++
	Dry synthetic agar .....	17	—	24	—	27	—
	Lima-bean agar .....	17	++	24	++	27	++
<i>Glomerella rufo-maculans</i> .....	Synthetic agar .....	17	+	24	—	27	—
	Dry synthetic agar .....	17	++	21	++	27	++
<i>Plowrightia morbosa</i> .....	Synthetic agar .....	17	++	21	++	27	++
	Dry synthetic agar .....	17	++	21	++	27	++
<i>Venturia inaequalis</i> .....	Synthetic agar .....	17	++	21	++	27	++
	Dry synthetic agar .....	17	++	24	++	27	++
<i>Cephalothecium roseum</i> .....	Synthetic agar .....	17	++	21	++	27	++
	Dry synthetic agar .....	17	—	21	—	27	—
<i>Colletotrichum Lindemulhianum</i> .....	Synthetic agar .....	17	—	21	—	27	—
	Dry synthetic agar .....	17	++	21	++	27	++
	Lima-bean agar .....	17	++	21	++	27	++
<i>Ascochyta colorata</i> .....	Dry Lima-bean agar .....	17	++	21	++	27	++
	Synthetic agar .....	17	++	21	++	27	++
	Dry synthetic agar .....	17	++	24	++	27	++
	Lima-bean agar .....	17	++	24	++	27	++
<i>Phytophthora oenothera</i> .....	Dry Lima-bean agar .....	17	++	24	++	27	++
	Lima-bean agar .....	17	++	28	++	30	++
<i>Pseudomonas campestris</i> .....	Dry Lima-bean agar .....	17	—	28	—	30	—
	Plain-agar slants .....	17	—	2	—	30	—
<i>Bacillus melonis</i> .....	Dry cotton wads .....	17	++	2	++	30	++
	Plain-agar slants .....	17	++	2	++	30	++
<i>Actinomyces chromogenus</i> ( <i>Oospora scab-</i> <i>tes</i> ) .....	Dry cotton wads .....	17	—	2	—	30	—
	Plain-agar slants .....	17	—	2	—	30	—
<i>Actinomyces bonis</i> .....	Dry cotton wads .....	17	—	2	—	30	—
	Plain-agar slants .....	17	++	2	++	30	++
<i>Actinomyces chromogenus</i> .....	Dry cotton wads .....	17	++	2	++	30	++
	Plain-agar slants .....	17	++	2	++	30	++
<i>Bacillus typhosus</i> .....	Dry cotton wads .....	17	+	2	+	30	+
	Plain-agar slants .....	17	++	2	++	30	++
	Dry cotton wads .....	17	—	2	—	30	—

If the results of the exposures of these organisms to low temperature are summarized, it will be noted that five fungi, *Sclerotinia cinerea*, *Cephalothecium roseum*, *Glomerella rufomaculans*, *Venturia inequalis*, and *Ascochyta colorata*, lived over winter under all conditions of exposure; while four others, *Alternaria solani*, *Cylindrosporium pomi*, *Plowrightia morbosa*, and *Phytophthora omnivora*, lived over on some media but not on others. One fungus, *Fusarium* sp. of conifers, succumbed to the low temperature, while two others, *Colletotrichum Lindemuthianum* and *Sphaeropsis malorum*, were so weak that only under very favorable conditions would they respond to fresh media. Only two of the six kinds of bacteria exposed can be safely said to have survived—*Bacillus melonis* and *Actinomyces chromogenus*. Transfers from exposed cultures of *B. melonis* were found to agree in all distinctive characters with those given by Giddings. It is to be noted that this organism forms no spores. The growth of transfers from exposed cultures of *Actinomyces chromogenus* was very characteristic and hardly mistakable for any other organism. In regard to the other bacterial cultures, it may be said that they were more or less contaminated during the exposure; and although some of the transfers from them resemble the original growth, this was not well enough marked to prevent all suspicion. On the whole, the various organisms seem to withstand exposure better in a dry condition than when food and moisture are present.

Thinking that some of the organisms might die from natural causes other than the exposure to low temperature, the author retained part of the culture made for this test indoors as a check. They were kept in a cool room (14 to 20° C.) throughout the winter and tested for vitality late in March, 1914. In practically every case these cultures were living at that time, and no organism given in Table III can be said to have died otherwise than by exposure to low temperature.

No entirely satisfactory explanation has been offered as yet of the changes which take place in fungi and bacteria during or after exposure to extreme cold. The results obtained by the author throw little or no light on the manner of the freezing nor on the subsequent death. The present work is a record of the fact that certain fungi and bacteria are able to withstand extreme cold, while others succumb to it, but does not attempt to advance any theory as to the internal changes which contribute to the weakening or death of the organisms thus tested.

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### EFFECT OF COLD-STORAGE TEMPERATURES UPON THE MEDITERRANEAN FRUIT FLY

By F. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant, Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

#### INTRODUCTION

Since the introduction of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) into the Hawaiian Islands and the subsequent quarantines against Hawaiian fruits, the problem of the fruit grower in these islands has been how to use his fruit to advantage at home. Many host fruits of the fruit fly are ruined long before they are suitable for either the table or storage. There are, however, other fruits, such as the avocado (*Persea gratissima*) and certain varieties of mangos (*Mangifera indica*) and star-apples (*Chrysophyllum cainito*), which, while often becoming too badly infested to be of use if left to ripen normally upon the tree, become infested so late in their development that they may be preserved for commerce if they respond favorably to cold storage, and if such cold storage kills whatever stages of the fruit fly may be present in the fruit when picked.

The experimental work reported in this paper was undertaken primarily with the hope that it would be an aid in solving the discouraging problems of the local horticulturists. But whatever its value in this direction, it now appears that the results may be of much greater commercial importance in defining the conditions under which cold-storage temperatures will kill the fruit fly in stored fruits, thus rendering them free from danger as transporters of this pest from one country to another or even from one infested district to another in host fruits.

#### HISTORICAL REVIEW

Cold-storage temperatures have been used in economic entomology in the past more to suspend insect activity than to cause death, except in the case of the Mediterranean fruit-fly work in Australia and Africa. The first practical use of cold-storage temperatures known to the writers was made by the manager of a large storage-warehouse company of Washington, D. C., in an attempt to find a safe method of protecting clothing from insect ravages during the warmer period of the year. At



the suggestion and with the assistance of Dr. L. O. Howard experiments were carried on to determine the effect of cold-storage temperatures upon still other insects affecting stored goods. Dr. Howard (1),<sup>1</sup> in a paper read before the eighth annual meeting of the Association of Economic Entomologists in 1896, discussed for the first time in professional entomological literature the important use to which cold-storage temperatures may be put in controlling insects. In 1905 Duvel (2), while investigating the storage of cowpeas (*Vigna sinensis*), found that storage at 32° to 34° F. was entirely practicable and economical in combating the common bean weevil (*Bruchus oblectus*), the cowpea weevil (*Bruchus chinensis*), and the four-spotted bean weevil (*Bruchus quadrimaculatus*).

While the work referred to above was carried on primarily to safeguard produce and stored goods from attack during certain periods when pests are active, experiments to determine the effect of cold-storage temperatures upon the Mediterranean fruit fly have been undertaken with the object of killing the various stages within the fruit. The interest in this work in Africa and Australia has grown out of the fact that the growers have sought for their surplus fruit markets in northern Europe, England, and North America, and even in South America, China, and the Hawaiian Islands. To reach these markets their fruits must be in transit a sufficiently long time for infestations overlooked at the packing houses to cause considerable decay unless the cold-storage temperature to which the fruit is subjected en route either suspends or kills chance cases of infestation.

In 1906, Fuller (3) recorded the resistance of fruit-fly larvæ in a certain lot of peaches in Natal to 40° F. for 124 days. The writers question the accuracy of this statement, as they have been unable at this temperature to keep larvæ or eggs alive for more than 22 days, in tests covering several thousand larvæ and eggs (see Table I). Fuller believes from his observation that cold storage as a method of substitution for quarantines involves considerable risk.

Lounsbury (4) states in 1907 that experiments conducted by him in South Africa indicate that a temperature of 38° to 40°, continued for three weeks, is sufficient to insure the death of all fruit-fly larvæ in infested fruit, that two weeks at such a temperature causes considerable mortality, and that one week is thoroughly ineffective. In 1908, in a second paper (6), he records no living larvæ among 511 specimens found in peaches held for 21 and 27 days at 38° to 40°. It is his belief that the storage temperature necessary for the preservation of fruit in transit from Africa to countries of the Northern Hemisphere and to America is amply low to effect the extinction of all life in larvæ and eggs of the fruit fly contained within it.

Hooper (5) recorded in 1907 in West Australia that he had found that larvæ and eggs of the fruit fly could not resist temperatures ranging from

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<sup>1</sup> Reference is made by number to "Literature cited, p. 665-666.

33° to 35° for more than 15 days, and advised that fruit kept within this range of temperature for three weeks would be perfectly free from living forms. His report indicates that the work was done carefully.

The work of Wilcox and Hunn (7) in 1914 has shown that such semi-tropical host fruits as the star-apple, fig (*Ficus* spp.), papaya (*Carica papaya*), mango, and avocado withstand without injury to texture or flavor a temperature slightly above 32° for from 27 days in the case of papaya to two months in the case of the avocado. Such periods at 32° are well above the margin of safety for complete mortality of the larvæ and eggs of the fruit fly.

#### EXPERIMENTAL WORK

In determining the effect of cold-storage temperatures upon the eggs and larvæ of the Mediterranean fruit fly, the writers have been fortunate in securing the cooperation of an ice company during 1913 and of an electric company during 1914 and 1915. At the cold-storage plants of these companies there were to be had all the facilities found in modern, well-regulated cold-storage plants. While an abundance of fruit-fly material is to be had in and about Honolulu, the writers have preferred in their work to infest in the insectary host fruits known to be previously free from attack. As no such fruits can be found in Hawaii under natural conditions, apples (*Malus* spp.) from California were used. These fruits were suspended for several hours in jars containing several hundred ovipositing fruit flies and then removed and held in the insectary for the number of days which experience had shown was necessary for the flies within to reach the stages desired for experiment. In this way larger amounts of material in definite stages could be used at one time than otherwise. While much of the data recorded in Table I was secured from fruit flies in apples, a sufficient amount, including observations on many thousands of eggs and larvæ, has been secured from fruit flies in peaches and kamani nuts (*Terminalia catappa*), as checks, to prove that there is no probability that the nature of the host fruit affects the action of temperatures.

No examination of material to determine the effect of various temperatures was made until the host fruits had been removed from storage from 24 to 48 hours. By placing the host fruits within storage the eggs and larvæ were under normal conditions. On examination the eggs were dissected out of the punctures and placed in moist chambers where all that hatched might be recorded. Larvæ found torpid though normal in color on examination within 24 to 48 hours after removal from storage invariably failed to resume activity.

#### THE EGG

No eggs hatch in cold storage if held at temperatures below 50° F.

A temperature of 32° proved quickly fatal to eggs. A total of 6,747 eggs were under observation. No eggs hatched upon removal from

storage after the ninth day of refrigeration. Only one egg hatched on the ninth day, and but 2 out of 2,327 removed on the seventh, eighth and ninth days. After the tenth to fifteenth days of refrigeration, 2,221 eggs were removed to warmer temperature, but none hatched. Mortality increased rapidly after the fourth day of refrigeration; thus, on the fifth day only 15 out of 735 eggs hatched. (See Table I.)

TABLE I.—Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly

Number of days in cold storage.	Temperature of storage room	Eggs		Larvæ.					
		Number under observation	Number hatching after removal from storage.	First instar		Second instar.		Third instar.	
				Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	° F.								
1.....	32	81	81	.....	.....	252	40	33	7
2.....	32	528	520	94	0	403	9	53	2
3.....	32	150	135	37	1	226	15	16	75
4.....	32	336	216	285	26	152	0	101	3
5.....	32	735	15	196	202	71	175	.....	.....
6.....	32	469	12	26	165	18	50	105	10
7.....	32	659	1	11	454	14	64	135	132
8.....	32	834	0	2	845	20	423	38	200
9.....	32	734	1	0	339	11	473	20	429
10.....	32	.....	.....	0	701	0	257	.....	.....
11.....	32	635	0	0	450	0	332	6	374
12.....	32	887	0	0	440	0	493	0	157
13.....	32	.....	.....	0	355	0	276	0	173
14.....	32	699	0	0	273	0	248	0	152
15.....	32	.....	.....	.....	.....	0	262	0	144
2.....	32-33	.....	.....	86	0	78	0	3	0
3.....	32-33	.....	.....	154	1	146	2	89	0
4.....	32-33	.....	.....	46	0	73	0	32	0
5.....	32-33	.....	.....	96	0	39	0	30	0
6.....	32-33	152	23	279	7	8	1	24	0
7.....	32-33	.....	.....	31	1	16	11	9	0
8.....	32-33	401	5	35	163	3	27	10	16
9.....	32-33	.....	.....	0	169	0	167	2	14
10.....	32-33	357	0	2	179	0	110	0	31
12.....	32-33	784	0	0	880	0	86	0	35
13.....	32-33	900	0	0	637	0	35	0	2
14.....	32-33	1,001	0	0	425	0	42	0	28
15.....	32-33	1,121	0	0	255	.....	.....	.....	.....
16.....	32-33	312	0	0	519	0	43	.....	.....
17.....	32-33	.....	.....	0	143	0	29	0	3
3.....	33-34	.....	.....	60	0	94	0	55	0
4.....	33-34	.....	.....	108	2	107	2	68	0
5.....	33-34	.....	.....	42	26	79	28	.....	.....
6.....	33-34	.....	.....	68	32	286	169	8	5
7.....	33-34	.....	.....	75	20	81	100	55	1
8.....	33-34	300	45	46	20	35	175	51	48
9.....	34-34	500	0	38	207	48	456	31	189
10.....	33-34	541	0	4	1,446	32	296	0	48
11.....	33-34	.....	.....	0	72	0	314	4	126
12.....	33-34	358	0	1	215	0	509	0	48
13.....	33-34	.....	.....	2	632	0	385	0	4

**TABLE I.**—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly—Continued*

Number of days in cold storage.	Temperature of storage room.	Eggs		Larvæ.					
		Number under observation.	Number hatching after removal from storage.	First instar		Second instar		Third instar.	
				Number alive	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	<i>° F.</i>								
14.....	33-34	1,035	0	0	76	0	245	0	49
15.....	33-34	746	0	0	710	0	301	3	154
16.....	33-34	1,058	0	1	763	0	65	0	53
17.....	33-34	513	0	0	521	0	45	0	134
18.....	33-34	1,000	0	0	514	0	46	.....	.....
19.....	33-34	.....	.....	0	221	0	67	0	18
8.....	34-36	.....	.....	.....	.....	0	11	7	170
9.....	34-36	.....	.....	.....	.....	0	21	1	176
10.....	34-36	.....	.....	0	44	0	8	5	321
11.....	34-36	236	0	0	192	0	60	0	225
12.....	34-36	.....	.....	0	74	0	138	4	399
13.....	34-36	241	0	.....	.....	0	84	0	436
14.....	34-36	.....	.....	0	111	0	19	0	354
15.....	34-36	.....	.....	0	42	0	6	0	158
2.....	36	167	131	.....	.....	120	5	242	2
3.....	36	281	261	166	3	261	1	260	6
4.....	36	419	419	127	2	245	4	180	22
5.....	36	433	405	288	2	473	25	256	24
6.....	36	365	254	75	57	334	12	158	77
7.....	36	184	150	28	142	147	43	62	157
8.....	36	454	264	1	382	0	323	33	363
9.....	36	858	335	1	475	0	300	2	402
10.....	36	301	27	0	494	0	385	0	160
11.....	36	652	2	0	588	0	437	0	186
12.....	36	728	0	0	670	0	858	0	213
13.....	36	534	0	0	504	0	91	0	364
14.....	36	463	0	0	443	0	54	1	261
15.....	36	568	0	0	573	0	22	1	198
16.....	36	480	0	.....	.....	0	38	0	251
17.....	36	532	0	.....	.....	.....	.....	.....	.....
3.....	36-40	.....	.....	.....	.....	42	2	.....	.....
4.....	36-40	.....	.....	.....	.....	127	46	.....	.....
5.....	36-40	.....	.....	.....	.....	123	3	.....	.....
6.....	36-40	.....	.....	.....	.....	127	25	.....	.....
7.....	36-40	.....	.....	.....	.....	18	94	.....	.....
8.....	36-40	.....	.....	.....	.....	0	13	60	258
9.....	36-40	136	0	.....	.....	0	25	3	112
10.....	36-40	128	0	.....	.....	.....	.....	.....	.....
11.....	36-40	125	0	0	102	0	18	0	275
12.....	36-40	122	0	0	23	0	12	0	256
13.....	36-40	.....	.....	.....	.....	0	25	0	352
14.....	36-40	185	0	0	32	0	275	0	522
15.....	36-40	.....	.....	.....	.....	0	218	0	163
16.....	36-40	.....	.....	0	48	0	69	0	324
17.....	36-40	106	0	.....	.....	0	131	.....	.....
18.....	36-40	.....	.....	0	118	0	18	0	97
19.....	36-40	210	0	.....	.....	.....	.....	.....	.....
20.....	36-40	.....	.....	0	16	0	64	.....	.....

TABLE I.—Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
		Number under observation.	Number hatching after removal from storage.	First instar.		Second instar.		Third instar.	
				Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	° F.								
10.....	38-40	.....	.....	38	8	19	8	10	1
12.....	38-40	.....	.....	4	25	26	19	36	6
13.....	38-40	.....	.....	3	60	15	0	.....	.....
14.....	38-40	.....	.....	0	36	17	40	.....	.....
15.....	38-40	.....	.....	15	46	5	24	.....	.....
16.....	38-40	.....	.....	0	99	14	148	1	25
20.....	38-40	.....	.....	0	42	0	39	4	3
23.....	38-40	.....	.....	0	43	0	84	.....	.....
25.....	38-40	.....	.....	0	18	0	133	0	1
28.....	38-40	.....	.....	0	33	0	27	0	9
30.....	38-40	.....	.....	0	44	.....	.....	.....	.....
2.....	40-45	12	12	.....	.....	.....	.....	.....	.....
3.....	40-45	55	19	.....	.....	.....	.....	.....	.....
4.....	40-45	26	0	.....	.....	.....	.....	.....	.....
5.....	40-45	8	3	.....	.....	.....	.....	.....	.....
6.....	40-45	16	12	.....	.....	.....	.....	.....	.....
8.....	40-45	14	7	.....	.....	.....	.....	.....	.....
9.....	40-45	31	17	.....	.....	.....	.....	.....	.....
11.....	40-45	14	1	.....	.....	.....	.....	.....	.....
14.....	40-45	31	1	.....	.....	.....	.....	.....	.....
15.....	40-45	30	0	.....	.....	.....	.....	.....	.....
17.....	40-45	26	6	.....	.....	.....	.....	.....	.....
19.....	40-45	21	0	.....	.....	37	34	80	56
20.....	40-45	67	2	.....	.....	79	79	138	135
21.....	40-45	127	0	.....	.....	107	130	187	103
22.....	40-45	50	0	.....	.....	.....	.....	.....	.....
23.....	40-45	15	0	.....	.....	92	97	160	226
24.....	40-45	21	0	.....	.....	68	125	125	220
25.....	40-45	38	0	.....	.....	14	281	89	88
26.....	40-45	.....	.....	.....	.....	30	95	106	320
28.....	40-45	.....	.....	.....	.....	0	9	27	208
29.....	40-45	.....	.....	.....	.....	1	131	57	112
31.....	40-45	.....	.....	.....	.....	0	161	8	201
32.....	40-45	.....	.....	.....	.....	0	8	4	139
33.....	40-45	.....	.....	.....	.....	0	290	5	318
36.....	40-45	.....	.....	.....	.....	0	218	7	397
37.....	40-45	.....	.....	.....	.....	0	345	3	393
38.....	40-45	.....	.....	.....	.....	0	204	7	377
39.....	40-45	.....	.....	.....	.....	0	42	1	385
40.....	40-45	.....	.....	.....	.....	0	84	0	401
41.....	40-45	.....	.....	.....	.....	0	112	2	330
42.....	40-45	.....	.....	.....	.....	0	92	0	292
44.....	40-45	.....	.....	.....	.....	0	39	0	200
45.....	40-45	.....	.....	.....	.....	0	36	1	689
46.....	40-45	.....	.....	.....	.....	0	23	0	476

Temperatures ranging from 32° to 33° proved equally fatal, the effect on 5,055 eggs being practically identical with that recorded for an even 32° F. Thus, no eggs hatched from batches removed between the ninth

and sixteenth days of refrigeration, although 4,475 were under observation. Only 5 eggs hatched out of 401 removed on the eighth day, and 23 out of 152 removed on the sixth day.

Temperatures ranging from 33° to 34° proved fatal after the eighth day; 45 eggs out of 300 removed on the eighth day hatched. No eggs hatched out of 6,051 removed between the ninth and eighteenth days of refrigeration.

At 34° to 36° eggs were examined only on the eleventh and thirteenth days of refrigeration. No eggs hatched out of 236 and 241 removed after these periods of refrigeration.

All the eggs subjected to a temperature of 36° were not killed until after the eleventh day of refrigeration. Out of 652 eggs removed from storage on the eleventh day, 2 hatched; and out of 301 eggs removed after 10 days, 27 hatched. No eggs hatched out of 3,305 removed after from 12 to 17 days of refrigeration. No appreciable mortality occurred at this temperature until after one week.

No eggs held at 36° to 40° were examined until the ninth day of refrigeration. Out of 1,012 eggs removed in small batches daily between the ninth and nineteenth days of refrigeration, none hatched.

Only 602 eggs were used for refrigeration at 40° to 45°. No eggs hatched after a refrigeration of 21 days. Two eggs out of 67 refrigerated for 20 days hatched on removal to the laboratory. No eggs hatched of those removed after 21 to 25 days of refrigeration.

#### THE LARVA

Larvæ in the third instar proved more resistant to cold than larvæ in the first and second; and all instars are generally more resistant to low temperatures than are the eggs. (See Table I.)

A temperature of 32° F. was found fatal to larvæ of the first instar after the eighth day of refrigeration; 2,558 larvæ removed after refrigeration from 9 to 14 days were found to be dead. The data in Table I show that 2 out of 845 were alive on the eighth day of refrigeration and only 11 out of 454 on the seventh day. This temperature did not appear to affect the first-stage larvæ appreciably until after the fifth day of refrigeration. Larvæ of the second instar failed to live after the ninth day, and very few lived that long; but 11 out of 473 and 20 out of 423, respectively, were alive after the eighth and ninth days of refrigeration. All of 1,868 second-instar larvæ were found dead on removal from storage after the tenth to fifteenth days of refrigeration. Only 6 out of 332 larvæ of the third instar were alive on the eleventh day of refrigeration; 626 larvæ removed after 12 to 15 days of refrigeration were found dead.

A temperature of 32° to 33° had practically the same effect upon 5,352 larvæ as did 32°.

Temperatures ranging from 33° to 34° did not prove entirely fatal to the first-instar larvæ until the seventeenth day of refrigeration; one larva out of 763 was alive on the sixteenth day. This was very exceptional and demonstrates the value of using an abundance of material and of continuing examinations after all larvæ seem to have been killed. Only 4 out of 1,446 were alive after 10 days of refrigeration; 1 out of 215 after 12 days, and 2 out of 632 after the thirteenth day of refrigeration. First-instar larvæ to the number of 1,256, removed after the seventeenth, eighteenth, and nineteenth days of refrigeration, were all dead. No second-instar larvæ subjected to 33° to 34° were found alive after the tenth day of refrigeration; 1,997 removed after 11 to 19 days of refrigeration were all dead. A few third-instar larvæ subjected to 33° to 34° lived until the fifteenth day of refrigeration, but none for a longer time. After the ninth day no larvæ were found alive, except during the examinations made after the eleventh and the fifteenth days of refrigeration, when 4 out of 126 and 3 out of 154, respectively, were found alive. A study of the data in Table I shows that a temperature of 34° to 36° had practically the same effect upon 1,615 larvæ as did that of 33° to 34°.

A temperature of 36° proved fatal to first-instar larvæ after the tenth day. After the ninth day of refrigeration 1 out of 476 was found alive. No living first-instar larvæ out of 3,272 were found alive after refrigeration from 10 to 15 days. The mortality at this temperature among first-instar larvæ became very noticeable after the sixth day of refrigeration, when 57 out of 132 larvæ were found dead. No second-instar larvæ were found alive after the eighth day of refrigeration; thus, all of 2,508 removed after refrigeration from 8 to 16 days were found dead. No third-instar larva was found alive after the ninth day of refrigeration, except on the fourteenth and fifteenth days, when 1 living larva was found out of 262 and 199 larvæ examined. After the ninth day but 2 out of 404 larvæ were found alive.

Temperature, 36° to 40° F.: No examinations were made to determine the effect of this temperature on the first-instar larvæ until after the tenth day of refrigeration. Of 339 larvæ removed after refrigeration from 11 to 20 days, none was alive. No living second-instar larva was found alive after the eighth day of refrigeration; after the seventh day 18 out of 112 were found alive. All of 868 second-instar larvæ removed after refrigeration from 8 to 20 days were dead. No living third-instar larva was found after refrigeration for 10 days, 3 out of 115 being alive after refrigeration for 9 days. All of 1,989 larvæ removed after refrigeration from 11 to 18 days were dead.

Temperature, 38° to 40° F.: All of 279 first-instar larvæ removed from storage after refrigeration from 16 to 30 days were dead, 15 out of 61 being alive after refrigeration for 15 days. No living second-stage larva was found after refrigeration from 20 to 28 days. No examina-

tions were made on the seventeenth, eighteenth, and nineteenth days; on the sixteenth day of refrigeration 14 out of 162 second-instar larvæ were alive. Third-instar larvæ were found alive after refrigeration for 20 days. No examinations were made between the twenty-first and twenty-fourth days, but no living third-instar larvæ were found during examinations of larvæ after the twenty-fifth and twenty-eighth days of refrigeration.

The warmest temperatures to which fruit flies were subjected ranged from 40° to 45°. Only larvæ of the second and third instars were used. One second-instar larva was alive on the twenty-ninth day, but no living second-instar larvæ were found thereafter, although a total of 1,658 larvæ were examined after refrigeration from 31 to 46 days. One third-instar larva was alive on the forty-fifth day. All of 476 third-instar larvæ examined on the forty-sixth day of refrigeration were dead. More data at this temperature are desirable to fix the limit safely in so far as the mature larvæ are concerned. Fruit is not, however, held at such high temperature as 40° to 45° for periods sufficiently long to kill the fruit-fly larvæ; hence, the effect of these temperatures is of far less importance than that of temperatures ranging from 32° to 40°.

#### CONCLUSION

The data contained in this paper show that no eggs or larvæ of the Mediterranean fruit fly survived refrigeration at 40° to 45° F. for seven weeks, at 33° to 40° for three weeks, or at 32° to 33° for two weeks. They may lead to the modification of existing quarantines and encourage the refrigeration of fruit subject to fruit-fly attack. It seems reasonable to conclude that sooner or later the certification of properly refrigerated fruit will be practicable. When an association of fruit growers or a people find it financially worth while there is no reason why they can not operate a central refrigeration plant under the supervision of an official whose reputation shall be sufficient to guarantee all fruits sent out from the plant to be absolutely free from danger as carriers of the Mediterranean fruit fly.

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# BIOCHEMICAL COMPARISONS BETWEEN MATURE BEEF AND IMMATURE VEAL,<sup>1</sup>

By WILLIAM N. BERG,

*Biological Chemist, Pathological Division, Bureau of Animal Industry*

## INTRODUCTION

Several excellent treatises on dietetics contain statements to the effect that immature veal—i. e., veal that is about 3 weeks old or less—is unfit for human food, but these statements apparently are not based upon experimental data. At least, a search of the literature showed that too few workers have studied this subject. Certain European writers say that immature veal is bad because certain American laws forbid the sale of veal less than 3 or 4 weeks of age. Conversely, the American laws were based, to some extent, at least, upon European opinion. The desirability of further experimental work was very apparent several years ago to Drs. Melvin and Mohler, of the Bureau of Animal Industry, who started the present investigation.

The following quotations are typical of the existing literature on the subject:

Thompson (1909, p. 141):<sup>2</sup> Veal, especially when obtained from animals killed too young, is unusually tough, pale, dry, and indigestible; but when the animals are slaughtered at the ripe age, the meat is sometimes tender, and is regarded by many as nutritious. It differs considerably from beef in flavor, and contains more gelatin and water but less fat and protein. Veal broth is nutritious, and affords a wholesome variety in the dietary for the sick. When too much is given it may excite diarrhea. Veal is much more used for invalids in Germany than elsewhere, although it figures less conspicuously in hospital dietaries there now than formerly. Bauer declares it to be more digestible than beef, but Pavy says, referring to both veal and lamb, "they are meats that it is desirable to avoid, generally speaking, in case of dyspepsia," and this opinion is prevalent in America as well as in England.

Also (p. 420): The meat of very young animals should never be eaten, and the sale of young or "bob" veal two or three weeks old is prohibited by law. It is indigestible, innutritious, and readily decomposes.

Hutchinson states (1911, p. 67-68): Veal is believed to be somewhat difficult of digestion, a belief which is confirmed by experiment, for it required two and a half hours for its digestion, as compared with two hours for beef (Jessen). The difficulty of digesting veal is somewhat surprising, for the connective tissue, though abundant, is very easily changed into gelatin. It is believed by some that the explanation is to be found in the ease with which the fibers of veal elude the teeth on mastication.

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<sup>1</sup> The object of the present work was to ascertain whether the flesh of calves 3 weeks of age and under is or is not fit for human food. The work was begun in the spring of 1912 at the suggestion of Dr. John R. Mohler, then Chief of the Pathological Division, Bureau of Animal Industry, and continued with little interruption up to the fall of 1914. The writer is indebted to Dr. Mohler for his very effective interest in the work and for many valuable suggestions.

<sup>2</sup> Bibliographic citations in parentheses refer to "Literature cited," p. 708-711.

No experimental data on the digestibility of veal were found in the writings of Bauer (1885) and Pavy (1881), referred to by Thompson; there was nothing more than the statement that veal was not easily digested.

Although the above-mentioned work of Jessen (1883) was apparently done as accurately as the technic of that day permitted, it was far from conclusive, partly because the experiments were not numerous enough and partly because biochemical methods for accurately measuring the speed of digestion from one stage to another had not been developed. In fact, the fundamental data regarding the chemical nature of the digestive process and of the various digestion products of proteins were just then being studied. In the same volume with Jessen's work is one of the early works of Kühne and Chittenden (1883), describing the then little-known bodies resulting from the digestion of proteins.

Undoubtedly, the alleged indigestibility of veal was a belief perpetuated by repeated quotation either of experiments too old to be conclusive or of opinions expressed elsewhere.

#### WORK OF PREVIOUS INVESTIGATORS

With the exception of the works of Fish (1911; 1912; 1914), very little direct experimental work was found, although a careful search of the literature was made. An excellent discussion of the subject by Fish and other workers has been published by the American Veterinary Medical Association (1912). In his earlier work Fish obtained data on the amount of moisture in immature veal and in beef, also on the freezing point of the juice from the tissues and on the specific gravity of such juice. He conducted dietetic experiments in which 7 families of 20 persons of various ages received immature veal as part of their diet. The following extracts are from his reports:

All partook of the veal and appeared to relish it. None of the families reported any disturbance of any of the bodily functions; the health was apparently normal and each family was ready to receive a portion whenever another carcass was available. (1911, p. 139.)

The claim that the flesh of very young animals has a laxative effect upon human beings (Walley) has not been verified in the present experiments. (1912, p. 148.)

In a recent work Fish found that beef and immature veal digested with equal speed in pepsin-hydrochloric acid (1913, p. 64). This last observation is in accord with that of Langworthy and Holmes (unpublished), who found that both immature veal and market veal, when fed to men as part of their diet, have practically the same coefficient of digestibility as beef—i. e., 93 per cent.

Sparapani (1914) studied the toxicity, or the alleged toxicity, of fetal flesh. From his results he concluded that bovine fetal serum was less toxic than adult serum—i. e., more fetal serum was required to kill a rabbit than adult serum when injected intravenously.

## EXPERIMENTAL WORK

## MATERIALS

At convenient intervals a live calf, 7 days old or less, was obtained from a veterinarian in Washington, D. C., who procured the supply from farms near by. Forty-one calves were procured in this way. On 12 of these animals quantitative data were obtained; the rest of the material was used in the feeding experiments with cats. Each calf was inspected by a member of the staff of the Pathological Division. In every case, except veal sample 7, the calf purchased was found to be in good condition.

Immediately after the calf was killed, dressed, and quartered, the meat was trimmed from the bones. When the calf was intended for quantitative analytic work and for digestion experiments, care was taken to remove the muscles entire or nearly entire, so as to exclude bits of bone, tendon, etc. The whole muscles, free from adherent fat and the tough, tendinous ends, were placed in a wide-mouth 8-liter glass-stoppered bottle and kept in cold storage at or very near 1° C. (34° F.) until used.

When the calf was intended for feeding to the experimental cats, the meat was trimmed less carefully, so that adherent fat, small pieces of soft bone, etc., were included in the material stored. To this were added the liver, kidneys, spleen, lungs, and heart, all of which the cats received in their food (see p. 705). About 10 kgm. of muscle were obtained from each calf. A detailed record was made of the dates on which the calves were killed, etc., so that the age of the meat when used for the various purposes was always known.

Along with the analyses and digestions made on the veal, control determinations were made on beef. The greatest care was taken throughout the entire work to be certain that the data on beef and veal were obtained under identical conditions. Whenever a calf was killed and the veal was intended for comparative work with beef, 10 pounds of ordinary lean beef round steak were purchased in a market near by. No inquiries were made regarding the beef; it represented so much lean beef purchased at random. Soon after being brought to the laboratory the beef was carefully trimmed—i. e., fat and connective tissue were removed, leaving only the lean muscle tissue, with a few small specks of fat here and there. This was transferred to an 8-liter glass-stoppered wide-mouth bottle and kept until used in cold storage alongside the bottle containing the veal. The beef was numbered to correspond with the veal—i. e., beef sample 8 was the beef used for control work on veal sample 8.

Sometimes the comparative analyses and digestions were begun on veal and beef 1 day old—i. e., 1 day in storage—although the beef was really mature beef of unknown age. In some experiments the meats were

a month old, but in every case the age is given. Naturally, after the veal and beef had been stored for several weeks, they acquired "off odors." This was always recorded, but the meats were always used as if perfectly odorless. Veal intended for feeding to the cats was always boiled. None was rejected, no matter how unappetizing it might have been to human beings.

#### STANDARD SOLUTIONS AND APPARATUS

In the chemical work on the veal and beef the nitrogenous substances and the moisture content were studied. Together these constitute about 95 to 97 per cent of the weight of the meat, so that the chemical work, while not too detailed, gave information on practically all constituents except the lipins. For the large number of nitrogen determinations standard  $N/5$  sulphuric acid and sodium hydroxid were used. Although all the nitrogen determinations were comparative—i. e., on veal and beef at the same time and under the same conditions—the absolute value of the standard acid was determined with the greatest care. This was done by precipitating and weighing the barium sulphate obtained from a known volume of the acid, and as an independent check on these results the acid was also standardized against pure ammonium sulphate and against pure sodium carbonate. It is perhaps true that with biological material such as meat the limit of accuracy is soon reached if ordinary care is used, and nothing is gained by taking unnecessary precautions. But because the wholesomeness of immature veal is a subject of controversy it was thought especially advisable to take too many precautions throughout the work rather than too few.

The volumetric apparatus used was standardized either by the United States Bureau of Standards or in the laboratory. A set of standardized analytic weights, a carefully calibrated Greene barometer, and a standardized thermometer from the Physikalisch-Technische Reichsanstalt (Charlottenburg, Germany), were used.

#### ANALYTIC DATA ON IMMATURE VEAL AND MATURE BEEF

##### TOTAL NITROGEN

The total nitrogen was determined on seven portions of each sample of beef and veal, of which three were made on the fresh meat, two on meat dried over sulphuric acid in vacuo at room temperature for two weeks, and two on portions dried for 12 hours at 95° C. in the hot-water oven.

No nitrogen determinations were made on veal samples 1 and 2—i. e., the first two calves—and the corresponding mature-beef samples. On veal and beef samples 3, 4, 5, 6, and 7 nitrogen determinations were made as just described. On veal and beef samples 8, 9, 10, 11, and 12

determinations were made as before, except that no portions of fresh meat were weighed for the direct determination of total nitrogen. Portions of 25 gm. each were weighed into suitable flasks and hydrolyzed by boiling with hydrochloric acid. After diluting to 250 c. c., two portions of 25 c. c. each, corresponding to 2.5 gm. of fresh meat, were pipetted into Kjeldahl flasks and the determination carried out as usual (see p. 678). In this way duplicate determinations were made on veal samples 8, 9, 10, and 11 and a single determination on sample 12. Duplicates were obtained on beef samples 8 and 11; on beef sample 10 four determinations were made and averaged, as the first two were not close enough; on beef sample 12 one determination was made. There was no beef sample 9. Veal sample 9 was compared with skim milk (skim-milk sample 2) which contained 5.29 mgm. of total nitrogen per gram of skim milk, or 0.529 per cent. Veal sample 5 was compared with beef sample 5 in some experiments and with skim-milk sample 1 in others—this contained 5.74 mgm. of total nitrogen per gram of skim milk.

All determinations of nitrogen were made by the usual Kjeldahl method, using metallic mercury, potassium sulphid, etc. Shortly after the appearance of the results of Trescot (1913), potassium sulphate was used in addition to the mercury, to assist in the oxidation. At first Congo red was used as indicator; later this was replaced by alizarin sulphonate.

The results for total nitrogen are summarized in Table I. It is apparent that the differences in nitrogen content between immature veal and mature beef are slight. The higher moisture content of the veal probably accounts for the slightly lower average figure, 3.14 per cent, as compared with 3.48 per cent for beef. The averages for the meats dried in vacuo are practically identical. For the meats dried in the hot-water oven, the average value for the veal, 14.08 per cent, is higher than that for the beef, probably because the veal dried more thoroughly—i. e., the average moisture in veal dried in vacuo was 77.08 per cent; in the hot-water oven, 77.54 per cent (see p. 683, moisture figures). The difference between the two figures for beef was not so great, the average for beef dried in vacuo being 74.18 per cent and in the hot-water oven 74.10 per cent.

TABLE I.—Percentage of total nitrogen in meat

Calf No.	Age of calf when killed.	Fresh.		Dried in vacuum desiccator.		Dried in hot-water oven.	
		Beef.	Veal.	Beef.	Veal.	Beef.	Veal.
	Days.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
3.....	7	3.45	3.33	12.67	14.03	11.78	14.65
4.....	5	3.49	3.18	13.56	13.86	13.65	14.03
5.....	6	3.51	3.24	14.62	13.04	14.26	13.37
6.....	5	3.60	3.00	13.23	13.26	13.42	13.60
7.....	5	3.59	3.40	14.40	14.41	14.47	15.12
8.....	3	3.53	2.95	12.60	13.55	13.52	13.74
9.....	7	(a)	3.12				
10.....	4	3.34	2.97	13.13	13.50	13.78	14.16
11.....	4	3.43	3.17	13.82	13.58	13.92	13.76
12.....	4	3.38	3.07	13.49	13.61	13.67	14.25
Average.....		3.48	3.14	13.50	13.65	13.61	14.08
Number of determinations averaged.....		24	24	18	18	18	18

<sup>a</sup> Skim-milk sample 2 was used instead of beef (see p. 695).

The figures for total nitrogen in dried meats (last four columns of Table I) were calculated back to the fresh basis for comparison with the figures obtained directly on the same samples of fresh meat, with the average results given in Table II.

TABLE II.—Average percentage of total nitrogen in meat (dried meat calculated to fresh basis)

Meat	Fresh	Dried in vacuum desiccator.	Dried in hot-water oven.
	Per cent.	Per cent.	Per cent.
Beef.....	3.48	3.46	3.46
Veal.....	3.14	3.15	3.19

It is apparent from Table II that the meats lost no nitrogen during the drying. (For the method of drying, see p. 683.) Benedict and Manning (1905, p. 312) found that "these meats [beef, chicken], therefore, after heating at 100° in a water oven lost from 4 to 7 per cent of the total nitrogen present." They quote similar observations by other investigators. What is important in this connection is not the mere loss of a small amount of nitrogen, which could be easily replaced in a diet, but the possibility that the lost nitrogen was present in the form of volatile amines, as suggested by Atwater (1895, p. 43). Some amines are very poisonous, and the presence of even small amounts of such bodies in immature veal would constitute a valid objection to its use. Although looked for, losses of nitrogen in the dried-meat samples were not observed. There may be two reasons for this: (1) The meats used were not decom-

posed, and, therefore, amines resulting from decomposition were absent; (2) the temperature inside the hot-water oven varied from 93° to 95° in winter to 95° to 97° in summer, and meat dried for 12 hours in this manner was not decomposed.

Another method of looking for toxic bodies was used, the veal being fed to cats (see p. 703).

The results for beef, summarized in Tables I and II, are practically identical with those generally obtained by other investigators. Thus, Davis and Emmett (1914, p. 449) found 3.624 per cent of nitrogen in beef dried at 100° to 105° C. for 20 hours, the result being calculated to the fresh basis. Their values for total nitrogen in beef are practically the same as those for either beef or veal in Table I. They found that there was but very slight loss, if any, on drying the meats at 100° to 105° as compared with the value found by the vacuum method. Richardson and Scherubel (1908, p. 1552) obtained the following results for total nitrogen in 13 samples of fresh lean beef: Maximum, 3.65 per cent; minimum, 3.34 per cent; average, 3.49 per cent. It is to be noticed that all the figures for fresh beef in Table I lie between this maximum and minimum, and the averages in both are practically identical. These investigators state (p. 1551) that—

In nearly all the work on beef the muscular portion known as the "knuckle" to butchers was made use of on account of its size, uniformity in structure, and its freedom from fatty tissue. The knuckle is the group of muscles known as the Crural Triiceps to anatomists and consists of the Rectus Femoris, Vastus Externus, Vastus Internus, and Anterior Gracilis. It was desired to experiment primarily upon the lean portion of beef, and fatty matter and gristle was trimmed away as far as possible in the preparation of the samples for analysis.

#### EXTRACTIVE NITROGEN

Portions of freshly hashed beef and veal, each weighing 100 gm. were extracted by heating in flasks with 800 c. c. of distilled water. The heating lasted one hour in a boiling water bath. After cooling and weighing the flasks, sufficient water was added to bring the final volume up to 1,000 c. c. of water plus 100 gm. of fresh meat. The total nitrogen was determined in duplicate 100 c. c. portions of the filtrates. Beginning with beef and veal samples 7, whenever meat was boiled for digestion experiments, control portions were boiled for extractive nitrogen. It is obvious that in measuring the amount of nitrogen going into solution by the digestion of meat, it was desirable to know the quantity of soluble nitrogen originally present.

In 100 c. c. of filtrate corresponding approximately to 10 gm. of meat, the extractive nitrogen actually titrated was equivalent to about 15 c. c. *N*/5 acid. In calculating the amount of nitrogen corresponding to 100 c. c. of filtrate, allowance was made for the moisture present in the meat—i. e., if the meat contained 75 per cent of water, the 100 c. c. of filtrate treated corresponded to 100/1.075 of the total extractive nitrogen



present in 100 gm. of meat. In 15 duplicate determinations on two portions of the same filtrate obtained from beef and veal samples 3 to 8, the average difference between duplicates was 0.26 c. c. *N/5* acid; one set of duplicates on beef sample 4, in which the difference was 1.53 c. c. *N/5* acid, was not included in this average; but the average of these two was included in the results in Table III. The data on skim milk were obtained by using 600 gm. of skim milk instead of 100 gm. of meat, making the proper calculated allowances for the water in the milk. The details of the precipitation of the casein, etc., are given on p. 692.

The results for extractive nitrogen are summarized in Table III. The last column gives the number of days that elapsed between the killing of the calf and the boiling of the meat. During this time the veal was in cold storage. This, of course, is not true of the beef. The beef when purchased was in all probability obtained from an animal killed from 8 to 18 days before. After being brought from the market, the beef was stored with the veal. While sample 3 of veal used in experiment 14 was 8 days old when boiled, the corresponding sample 3 of beef can be said to have been stored for 8 days, but its age is not known. For this reason the comparison between the two is not exact. For some purposes it might have been desirable to kill a mature animal on the premises and store the beef immediately, as was done with the veal. But the principal object was a comparison of the veal with meat as purchased in the market.

TABLE III.—Percentage of extractive nitrogen in meat

Sample No	Beef	Veal	Experiment No	Age of meat when boiled.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Days.</i>
3.....	<i>a</i> 0.456	<i>a</i> 0.534	14	8
4.....	.433	.508	15, 16	1, 9
5.....	.437	.472	17, 18	7
5.....	<i>b</i> 0.364	.472	19	<i>c</i> 18
6.....	.473	.448	20, 21, 22	2, <i>d</i> 13, <i>e</i> 21
7.....	.433	.646	23	3
7.....	.693	<i>f</i> 1.526	24	<i>e</i> 33
8.....	.505	.520	26	8
8.....	.610	.520	25	<i>g</i> 31
9.....	<i>h</i> 0.615	.490	27	6
9.....	<i>h</i> 0.754	.539	28	21
10.....	.466	.553	30	19
10.....	.495	.645	31	<i>d</i> 28
11.....	.455	.519	32	19
12.....	.437	.496	34	8
Average.....	.491	.530	.....	.....
Determinations averaged.....	12	13	.....	.....

*a* Meat hashed, kept in cold storage till next day, then boiled. All other samples hashed and boiled same day. Veal sample 5, experiment 17, was hashed and boiled the same day calf 5 was killed.

*b* Figure for extractive nitrogen in skim-milk sample 1 omitted from average.

*c* Veal had an "off odor."

*d* Beef and veal had an "off odor."

*e* Beef and veal very poor, not fit to eat.

*f* Veal sample 7, calf had white scours, figure omitted from average.

*g* Veal had no odor. Beef had slight odor of hydrogen sulphid.

*h* Figures for skim-milk sample 2 omitted from average.

With the exception of veal sample 7, all of the calves purchased were in good condition. Calf sample 7 was known to have "white scours," or diarrhea. It was plainly a sick animal and was purposely obtained. A very young kitten gained considerable weight while utilizing veal sample 7, boiled, as its sole source of nitrogen (see p. 707). The high content of extractive nitrogen in veal sample 7, experiment 23, while comparatively fresh, and its very rapid autolysis, as indicated by its appearance and still higher extractive nitrogen content in experiment 24 a month later, are very striking. The four duplicates on veal and beef samples 7 were excellent.

Hansoulle (1910, p. 122), in his report on very young veal as food, quotes Fonsny to the effect that about 60 per cent of the dry matter in meat from very young calves consists of extractives and gelatin, materials which, while digestible, are not assimilable. Hansoulle also quotes the opinions of several directors of abattoirs in Belgium and France who regard very young veal as unfit for human food, but references to experimental work are not given. After veal sample 7 had been stored for over a month, the extractive nitrogen—i. e., nitrogen soluble in water near the boiling point—amounted to 44.9 per cent of the total nitrogen in the meat. But, obviously, this was exceptional, at least for the calves used in this work. It is possible that under the conditions observed by Hansoulle the veal deteriorated rapidly and justified his strong pronouncements on the unfitness of very young veal. The data in Table III have been obtained on calves 7 days old or less when killed, the meat of which had been stored at about 34° F. (1° C.) for varying lengths of time. The differences between the figures for beef and veal are much smaller than would be expected from the statements of various writers that the elimination of excretory nitrogen in very young calves is slow. Excluding the figure for veal sample 7 in experiment 24, the average extractive-nitrogen content in fresh beef is 0.491 per cent, and for fresh veal, 0.530 per cent, with no great variations from the average. If the figure for veal sample 7 be included, the averages are 0.491 per cent for beef and 0.601 per cent for veal. The figures for beef are essentially the same as those obtained by other workers.

Richardson and Scherubel (1908, p. 1527), in their studies on cold-storage beef, extracted 100-gm. portions of fresh beef with water until 1 liter of extract was obtained from each. Determinations of nitrogen in the various forms were made on 50 c. c. portions of the extract. By adding together their figures for the amount of nitrogen present as ammonia (method 2), albumoses, and meat bases in their cold-water extract, a figure is obtained which corresponds to the figures for extractive nitrogen in Table III. The term "extractive nitrogen" is used rather loosely here, as it includes all nitrogenous substances in meat which are soluble in water near the boiling point—i. e., proteoses, peptones, amino acids, ammonia, purin bases, etc. The slight loss of ammonia due to the

coagulation of the meat and the heating in water was not determined or allowed for in the calculations; it is too small. Richardson and Scherubel (p. 1552) obtained the following averages on cold-water extracts from 13 samples of fresh beef (see p. 674): Nitrogen present as ammonia 0.010 per cent; albumoses, 0.024 per cent; meat bases, 0.071 per cent. The total, 0.405 per cent, corresponds closely to the average of 0.491 per cent for beef in Table III. It is natural that the figure in Table III should be a trifle higher, as it includes data on both fresh and cold-storage beef. The storage temperature was practically the same as that used by Richardson and Scherubel—i. e., 2° to 4° C. (36° to 39° F.). It will be noticed in Table III that while the meats were in cold storage for the periods there indicated the extractive nitrogen increased very appreciably in beef samples 7, 8, and 10 and in veal samples 9 and 10. The same probably happened in beef and veal samples 3 to 6, but data were obtained on these only when fresh.

Similar increases in extractive nitrogen were noticed by Richardson and Scherubel (1909, p. 99) in their study of the changes taking place in beef stored at 2° to 4° C. In their samples proteolysis took place more slowly than in those of Table III, probably because, as they state (p. 101), "the knuckles (weight 7 to 8 pounds) were hung in a temperature of 2° to 4° C. immediately after slaughter and were allowed to remain there during the period when analyses were made, that is for 121 days."

The meat stored for use in the present work was cut into pieces not much larger than a hen's egg of good size. Undoubtedly this treatment permitted more active autolysis and bacterial decomposition than would have taken place had the veal and beef been stored in larger masses. As previously indicated, entire muscles were dissected from the veal quarters for the sake of uniformity of composition of the muscle tissue used for analysis, etc., necessitating the storage of comparatively small pieces of meat (see p. 669).

Emmett and Grindley (1909) found that in beef stored for 22 days at 33° to 35° F. (0.5 to 2° C.) the extractive nitrogen, contrary to expectations, did not increase, but a slight increase was noticed in beef stored under the same conditions for 43 days (p. 425). It is probable that one reason for this observation is to be found in their method of preparing cold-water extracts of beef for analysis. Portions of the experimental beef weighing 30 to 35 gm. were repeatedly extracted with cold water, and the extracts after filtration were diluted to 5 liters (Grindley and Emmett, 1905, p. 663). After removing coagulable nitrogen in a 200 c. c. portion of such a filtrate, corresponding to 1.2 gm. of meat, a further partition of nitrogen was made on the very small amounts of nitrogen remaining. The unavoidable errors in analytic work become proportionately large under such conditions, and the detection of slight changes in meat stored under good conditions for short periods of time becomes difficult.

Many investigations have been made on the behavior of beef when frozen, but such results are not exactly comparable with those obtained by the foregoing investigators nor by the writer on beef stored at or near 2° C.

It is obvious that the beef and veal used in this work underwent proteolysis during the storage periods to practically the same extent. The changes that took place in the beef are entirely comparable with those observed by others in beef stored under similar conditions. The slightly higher average content of extractive nitrogen in the veal (Table III) is not regarded as physiologically significant in the present consideration of the fitness of 1-week-old veal as food. The extractives of immature veal are the same as those of mature beef (Lindsay, 1911), and the slight quantitative difference found between the 10 "bob-veal" calves and their corresponding 10 samples of lean beef (summarized in Table III) do not warrant the inference that the tissues of the very young calf are loaded with unexcreted nitrogenous waste products.

#### AMINO NITROGEN IN MEAT EXTRACTIVES

The hot-water extracts of beef and veal used for the determination of extractive nitrogen were also used for the determination of amino nitrogen in the nitrogenous extractives present. The figures obtained were used as blanks in those digestion experiments in which the rate of digestion was measured by the rate of formation of amino nitrogen (see p. 696). Any marked differences between the figures for beef and those for veal might have led to the detection of significant differences in their composition.

Ten c. c. of filtrate, containing the extractives from not quite 0.5 gm. of beef or veal, were introduced into the Van Slyke amino-nitrogen apparatus and the amino nitrogen determined exactly as it was determined in the digestion experiments (see p. 680). The volume of gas measured was small, ranging from 1.9 to 5 c. c. The weight of nitrogen so obtained was calculated to 1 gm. of fresh meat, and this figure was divided by the weight of extractive nitrogen in 1 gm. of meat. The results are summarized in Table IV. In experiment 27 the digestibility of veal sample 9 was compared with that of skim milk instead of beef. The amino-nitrogen determination on skim-milk sample 2 was made on 10 c. c. of diluted skim milk containing 600 gm. of skim milk diluted to 1,000 c. c. which was used for other determinations (see p. 695). In this case the amino nitrogen was derived not only from the nonprotein extractives but from the proteins as well. The amino nitrogen obtained was calculated to 1 gm. of skim milk, and this figure was divided by the weight of extractive nitrogen found in 1 gm. of skim milk by the method described on p. 695.

TABLE IV.—Percentage of amino nitrogen in extractive nitrogen in beef and veal

Sample No	Experi- ment No.	Beef.	Veal.	Sample No	Experi- ment No.	Beef.	Veal.
		<i>Per cent.</i>	<i>Per cent.</i>			<i>Per cent.</i>	<i>Per cent.</i>
8.....	26	27	18	11.....	32	19	16
8.....	25	27	18	12.....	34	23	19
9.....	27	a 60	11				
10.....	30	22	19	Average .....		24	18
10.....	31	24	24				

a Figure for skim-milk sample 2; not included in the average (see p 695).

The figures for the percentage of amino nitrogen in Table IV were obtained by single determinations on each filtrate. Duplicates on veal sample 10 and skim-milk sample 2 agreed almost exactly, which is to be expected when small volumes of nitrogen gas are obtained in this determination. This, together with the comparatively large blank on the reagents, makes the experimental error in these determinations higher than in others. Nevertheless the data have been obtained on five different calves and their control samples of beef, and the uniformly higher amino-nitrogen content in the beef extractives is probably a correct indication of a slight difference between the beef and veal. The significance of this difference, if any, requires further work for its elucidation.

#### DISTRIBUTION OF NITROGEN IN BEEF AND VEAL HYDROLYZED BY HYDROCHLORIC ACID

**HYDROLYSIS.**—The beef and veal were hydrolyzed by boiling with hydrochloric acid in 300 c. c. Jena glass Erlenmeyer flasks provided with ground-in condenser tubes 100 cm. in length. Into a weighed flask 25 gm. of meat were weighed quickly to the nearest 0.1 gm. and the exact weight noted. Two such portions of beef and two of veal were weighed from large samples of the meats freshly hashed for several determinations. To each flask 175 c. c. of hydrochloric acid (1 : 1) were added. The ratio is 35 parts of 20 per cent hydrochloric acid to 1 of protein, found by Van Slyke (1912, p. 296) to hydrolyze proteins completely after boiling for 24 hours. In all the experiments except the first with beef and veal sample 8 the hydrolytic mixture was boiled for 24 hours. Beef and veal samples 8 were boiled for 24 and 48 hours, but no differences in the results were found. A small piece of broken glass added to the material in the flask prevented bumping. After boiling the required length of time the mixtures were cooled, transferred to 250 c. c. volumetric flasks, and diluted to the mark. Portions of these mixtures were used in the following determinations.

**TOTAL NITROGEN.**—From each of the four flasks 25 c. c. portions of the mixture, corresponding very nearly to 2.5 gm. of meat, were pipetted into Kjeldahl flasks and the total nitrogen determined. The results obtained in this way on beef and veal samples 8 to 12 have been given in Table I.

**AMMONIA.**—The Boussingault-Shaffer method, as described by Berg and Sherman (1905), was used for the determination of ammonia<sup>1</sup> in the hydrolytic mixture. The apparatus used was, in general, similar to that used by Van Slyke (1911, p. 21).

Fifty c. c. of the hydrolytic mixture, corresponding to 5 gm. of meat, were used. It was desired to know whether the general assumption that no nitrogen is carried over by the hydrochloric-acid distillate was correct or not. For this purpose the distillates from beef, whenever obtained, were transferred to the same Kjeldahl flask, while the distillates from veal were transferred to another. The total nitrogen was then estimated in the usual manner. Distillates corresponding to 25 gm. of beef and 35 gm. of immature veal yielded in both cases less than 0.2 c. c. of  $N/5$  nitrogen, indicating that none was lost during the distillations.

The distillation of the ammonia was carried out as usual for one hour in every case, during which time there appeared to be no splitting off of "cleavage ammonia," as numerous tests indicated.

In the hydrolytic mixtures obtained from beef and veal the ammonia nitrogen was about 7 per cent of the total nitrogen. Because of the small amount of ammonia actually distilled, corresponding to 5 gm. of fresh meat, or about 1 gm. of protein, the unavoidable errors in the analyses are proportionately large. The differences between six duplicates on beef and veal samples 8, 10, and 11 (Table V) varied from 0.04 to 1.33 per cent of the total nitrogen; average, 0.5 per cent. An idea of the limits of accuracy of this determination may be obtained by comparing the figures for ammonia nitrogen in casein by Van Slyke (1912, p. 297), who found 10.1 and 10.27 per cent, with those by Sherman and Gettler (1913), who found 10.0 per cent.

In order to be better able to compare the results for ammonia nitrogen, etc., in beef and veal with similar results by other workers, a sample of pure casein was hydrolyzed, using 5 gm. of casein instead of 25 gm. of fresh meat. The results obtained were: On casein hydrolyzed for 24 hours, 10.04 and 10.38 per cent, and for 48 hours, 10.55 and 10.81 per cent, of the total nitrogen present as ammonia, indicating that the technic used was essentially similar to that used by the above investigators (see p. 682).

**MELANIN NITROGEN.**—To the mixture remaining in the distillation flask after the removal of ammonia 3 c. c. of concentrated hydrochloric acid were added, the material transferred to a 100 c. c. volumetric flask, and diluted to the mark. This was then filtered into a second clean, dry 100 c. c. flask, and the nitrogen was determined in the melanin on the filter paper, corresponding to 5 gm. of meat, by the Kjeldahl method in the usual manner. To the figure so obtained there was added the amount of melanin nitrogen occasionally obtained by filtering the hydrolytic

<sup>1</sup> For excellent discussions of the various methods for determining ammonia, see Smith (1913); also Shulansky and Gies (1913).

mixture before any determinations were made. The filtrate was used in the determinations of amino nitrogen.

**AMINO NITROGEN.**—Van Slyke's (1912) apparatus and method were used in this determination in exactly the same manner for the hydrolytic mixtures, digestion mixtures, and control determinations on the reagents, leucin and pure casein. In every case the reaction between the reagents and the solution introduced into the apparatus was allowed to go on for exactly 20 minutes. Two, and sometimes three or four, determinations of amino nitrogen were made on every sample mentioned in Table V. The distribution of nitrogen was not studied in beef and veal samples 1 to 7. Two determinations on the same solution of hydrolyzed beef or veal generally differed by 2 per cent; thus, the figures obtained for veal sample 8 were 70.2 and 72.3 per cent of the total nitrogen present in the amino form. The average of these, 71.2 per cent, is the figure recorded in Table V. The extremes in this respect were: Beef sample 11 with 71.6, 74.6, and 75.8 per cent, a difference of 4.2 per cent between the highest and lowest figures, and beef sample 12 with 74.2, 74.4, and 74.7 per cent, the difference being 0.5 per cent. When the difference of 2 per cent was obtained with the first duplicates it was believed to be due to error in procedure. Accordingly, the determinations of the next sample, veal sample 9, were made with the greatest care but with no closer results. Numerous modifications of the method were tried without the desired result. A large number of results were obtained on veal sample 9 and skim-milk sample 2, all of which were low by several per cent and have been omitted from Tables V and XII. The fact that any deviation from the procedure used for beef and veal samples 8 gave uniformly low results led to its use without modification throughout the remainder of the work.

TABLE V.—*Distribution of nitrogen in beef and veal hydrolyzed by hydrochloric acid*

[Total nitrogen=100 per cent.]

Sample No.	Ammonia nitrogen	Amino nitrogen.	Nonamino nitrogen, by dif- ference.	Melanin nitrogen.	Experi- ment No.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
Beef 8 . . . . .	7.6	70.9	20.0	1.5	26
Veal 8 . . . . .	7.4	71.2	19.7	1.7	
Beef 10 . . . . .	7.1	74.5	17.6	.8	30
Veal 10 . . . . .	7.1	73.1	19.0	1.0	
Beef 11 . . . . .	6.8	74.0	19.4	.8	32
Veal 11 . . . . .	7.4	70.8	20.5	1.3	
Beef 12 . . . . .	7.5	74.4	17.6	.5	34
Veal 12 . . . . .	5.6	73.5	20.3	.6	
Average:					
Beef 8 to 12 . . . . .	7.2	73.4	18.6	.9	
Veal 8 to 12 . . . . .	6.9	72.1	19.9	1.1	
Beef 1.1 . . . . .	6.8	75.0	17.4	.8	33
Veal 1.1 . . . . .	6.7	75.1	17.1	1.1	
Casein 1 . . . . .	10.4	71.8	16.1	1.7	29
Casein, by Van Slyke . . . . .	10.1	72.1	16.1	1.8	

CONTROL DETERMINATIONS.—Control determinations on leucin and casein and later on tyrosin were made for the purpose of ascertaining whether errors in procedure were responsible for the unexpected differences between duplicates or whether the nature of the experimental material was such that interfering reactions made it practically impossible to obtain as close duplicates on so complex a mixture as hydrolyzed meat as can be obtained on certain pure amino acids. It is almost certain that the hydrolyzed meat contains a large variety of amino acids, some of which react quantitatively in five minutes; others require several hours; and no particular reaction time is favorable for all taken together. On this point the following quotations from the work of Van Slyke (1911) are of interest:

*Time required for different classes of amino derivatives to react quantitatively.* Amino groups in the  $\alpha$ -position to carboxyl, as in the natural amino-acids, react quantitatively in 5 minutes at 20°. The group in *lysine* requires one-half hour to react completely, *lysine* being the only natural amino-acid which requires more than 5 minutes. *Ammonia* and *methylamine* require 1.5–2 hours to react quantitatively. *Urea* requires 8 hours. . . . Amino groups in *purines* and *pyrimidines* require 2–5 hours at 20° (p. 191).

*Amino-acids which react abnormally with nitrous acid.* *Glycocoll* and *glycyl peptides*. *Glycyl-glycine*, unlike the other peptides, reacts not only with its free primary amino nitrogen, but also as Fischer and Koelker have shown, with a part of the secondary nitrogen in the peptid linking. This is doubtless connected with the peculiar behavior of *glycocoll* itself when treated with nitrous acid. It gives off not only nitrogen, but carbon dioxide and traces of some other gas, which is not absorbed by permanganate, indicating that decompositions deeper than the deamination occur. The behavior of *glycocoll* and *glycyl peptides* can be explained in three ways: . . . (p. 197) The gas measured is about 103 per cent of the theoretical volume of nitrogen . . . (p. 199).

In the determinations on hydrolyzed meat it was observed that almost invariably the nitrogen gas measured would diminish a few tenths of a cubic centimeter in volume, if the gas were passed back into the alkaline permanganate pipette and allowed to remain there overnight. Whether this was due to the *glycocoll* resulting from the hydrolysis of the different proteins in the meat or to other disturbing factors can not be stated. It is probable that the secondary reactions mentioned above take place when hydrolyzed meat reacts with nitrous acid for 20 minutes, and they contribute to the difficulty of obtaining very close duplicates.

For the control determinations on leucin a sample of Kahlbaum's synthetic leucin was used. This sample was dry and contained 96.4 per cent of the theoretical total nitrogen obtained by the Kjeldahl method, indicating the presence of a non-nitrogenous impurity. Six determinations on *N/10* leucin in 1 per cent (approximately) hydrochloric acid, made at various times throughout the work gave the following results: 95.4, 95.6, 95.3, 95.3, 96.1, and 96 per cent of the theoretical total nitrogen present as amino nitrogen; average, 95.6 per cent. One result, 94.4 per cent, obtained with exhausted permanganate in the absorption pipette,



was omitted from the average. Close duplicates on leucin and on the next control substance, casein, were obtained easily and by the identical methods that failed to produce as close duplicates on hydrolyzed meat.

The casein (casein sample 1) hydrolyzed for the control determinations was prepared in the laboratory in the usual manner, from separator skim milk. The dry protein contained 14.87 per cent of nitrogen and 0.10 per cent of ash. Although small amounts of impurities were probably present in this preparation, it compared favorably with those used by other workers. The hydrolysis of the casein, distillation of ammonia, and determination of amino nitrogen were carried out exactly as with hydrolyzed meat, except that 5 gm. of the dry casein were used instead of 25 gm. of meat. The following results were obtained: 71.37, 72.81 per cent (boiled for 24 hours), and 71.37, 71.73 per cent (boiled for 48 hours). The average of these four, 71.8 per cent, given in Table V, is very close to the figure (72.1 per cent) obtained by Van Slyke (1912, p. 297) and other investigators. The various determinations made with casein sample 1 indicate that the methods used were essentially correct and would yield close duplicates on materials to which they were applicable.

It was thought possible that the fats or their hydrolytic products might interfere with the amino-nitrogen determination, and for this reason determinations were made on beef and veal samples 1.1. These were dry, almost fat-free meat powders, prepared early in the work from beef and veal samples 1 by treating the hashed meats with alcohol and ether (see p. 685). The hydrolysis and determinations were made on these materials as usual, but no better duplicates were obtained. The figures for beef sample 1.1 were 74.1, 75.3, and 75.7 per cent; average, 75 per cent; for veal sample 1.1, 74.1, 74.1, 75.7, and 76.3 per cent; average, 75.1 per cent. The difference between the highest and the lowest figure for veal sample 1.1, 2.2 per cent, corresponds to a difference of 0.6 c. c. of nitrogen gas under the conditions of the determinations, in which the volume of gas actually measured was about 20 c. c.

A sample of tyrosin labeled "Tyrosin, pure, synthetic, Schuchardt" was also used for control determinations. It contained 1.66 per cent of moisture. Calculated to the dry basis the total nitrogen content by the Kjeldahl method was 93.5 per cent of the theoretical. The figures for amino nitrogen were 95.6, 96.0, and 95.3 per cent of the theoretical total; average, 95.6 per cent. In the first determination the gas after being measured was passed back into the absorption pipette, where it remained overnight. As usual, there was a slight diminution in volume—from 95.6 to 95.3 per cent.

It is believed that close duplicates on beef and veal have not been obtained, for reasons inherent in the material; the method used gave good results on comparatively pure leucin, casein, and tyrosin. The comparison between the amino-nitrogen content of beef and that of veal having been made under similar conditions, the data in Table V, although

possibly erroneous to the extent of 1 or 2 per cent, indicate that the differences between the mature beef and the immature veal are too slight to be significant.

**NONAMINO NITROGEN.**—"The difference between the Kjeldahl and  $\text{NH}_2$  determinations gives the nonamino ( $\text{NH}$ ) nitrogen. This includes one  $\text{NH}_2$  group, that of the guanidine nucleus of arginine, which does not react with nitrous acid . . ." (Van Slyke, 1912, p. 296).

#### PERCENTAGE OF WATER IN BEEF AND VEAL

Two 3-gm. portions of each meat, contained in porcelain crucibles, were dried in a vacuum desiccator and two similar portions in a hot-water jacketed oven.

**DRYING IN THE HOT-WATER JACKETED OVEN.**—The temperature of the interior of the oven ranged from  $93^\circ$  to  $95^\circ$  C. in winter to  $95^\circ$  to  $97^\circ$  C. in summer. The samples were transferred to the oven immediately after being weighed and were dried for 12 hours. A slow stream of clean dry air was passed through the drying chamber for several hours during the drying period, after which the crucibles were transferred to a desiccator, cooled, and weighed.

**DRYING IN THE VACUUM DESICCATOR.**—The samples of hashed beef and veal were transferred to a Hempel's desiccator; this was evacuated to about 85 mm. of mercury and the drying allowed to take place for two weeks at room temperature. During this time the sulphuric acid was changed once, and the desiccator was evacuated several times.

The dried samples, after being weighed, were transferred at convenient times to Kjeldahl flasks for nitrogen determinations except beef and veal samples 1 and 2. On these ash was determined by igniting the dried material. The results were: Beef sample 1, 1.16 per cent; beef sample 2, 1.10 per cent; veal sample 1, 1.14 per cent; veal sample 2, 1.14 per cent of ash. Calf 1 was 5 days old when killed; calf 2, 3 days. The ages of the others have been given in Table I. The results for water in beef and veal are summarized in Table VI.

TABLE VI.—Percentage of water in beef and veal

Sample No.	Dried in vacuum desiccator 2 weeks at room temperature.		Dried in water-jacketed oven 12 hours at $95\pm 2^\circ$ C.	
	Beef.	Veal.	Beef.	Veal.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1.....	73.25	76.83	73.58	77.10
2.....	73.59	78.74	74.03	79.83
3.....	72.98	77.13	71.49	76.37
4.....	75.28	77.39	74.84	77.39
5.....	76.46	74.38	75.01	75.79
6.....	72.69	76.12	73.63	77.60
7.....	75.13	78.01	75.12	77.98
8.....	72.54	77.76	71.35	77.93
10.....	74.48	77.42	75.45	78.38
11.....	75.11	76.86	75.99	77.05
12.....	74.43	77.20	74.66	77.54
Average of 22 determinations.....	74.18	77.08	74.10	77.54

Although care was taken to insure as uniform sampling as possible, the differences between duplicates varied from 0 per cent to 4.70 per cent—i. e., the figures for veal sample 11 were 77.05 and 77.05 per cent; for beef sample 8, 69 and 73.70 per cent. In every case the average of the duplicates is given in the table. The average of the 44 differences (there were 44 duplicates) was 0.92 per cent. Although theoretically simple, the determination of water in such material as meat is practically very difficult.<sup>1</sup> The results for beef and for veal are strictly comparable in so far as both sets were obtained under the same conditions, but they are not exact in the absolute sense. Had the samples been heated for more than 12 hours in the hot-water oven, the "moisture content" would have been higher, partly because more water would be driven off, and partly because other substances would volatilize, decompositions would begin, etc. Apparently, under the conditions of the determinations, errors which result from heating meat over 100° C. for long periods of time were obviated (Davis and Emmett, 1914).

The figures in Table VI for beef are similar to those obtained by other workers. Richardson and Scherubel (1908, p. 1527, 1552) found an average of 76.35 per cent of moisture in beef which had been dried to constant weight at 100° to 105° C. Grindley and Emmett (1905, p. 659) found 75.46 per cent of moisture in beef dried in a hot-water oven for a length of time not stated.

Obviously, the claim that immature veal ("bob veal") is more watery than beef finds little support in the data obtained, because the difference between the averages, about 3 per cent, is physiologically of no importance.

#### COMPARATIVE DIGESTIBILITY OF MATURE BEEF AND IMMATURE VEAL IN VITRO

In the following comparative measurements of the speed of proteolysis of beef and veal, an attempt was made to ascertain whether immature veal is more resistant to pepsin and trypsin than beef, as sometimes stated. Three separate methods were used, each of which has its advantages, disadvantages, and errors. In the first method the undigested meat was filtered at the end of the digestion period, dried, and weighed. In the second, nitrogen was estimated in portions of the digestive fluid from time to time, thereby giving an indication of the rate at which nitrogenous substances were going into solution. In the third, the rate of formation of amino nitrogen was estimated in portions of the digestive fluid, indicating the rate at which the amino-nitrogen groups interlocked in the polypeptids present were opened or separated by the trypsin and alkali.<sup>2</sup>

<sup>1</sup> For a discussion of the errors entering into this determination, see Benedict and Manning (1905).

<sup>2</sup> For discussions of the earlier work on artificial digestion, see Grindley, Mojonner, and Porter (1907, p. 61), and Berg (1909).

**SOLUTIONS.**—Digestions were made in 0.2 per cent hydrochloric-acid and in 0.5 per cent sodium-carbonate solutions.

**ENZYM PREPARATIONS.**—The following preparations, all in powder form, were used: .

**Pepsin 1:** A 100-gm. bottle of pepsin (1:3,000), Parke, Davis & Co.; purchased about May, 1912.

**Pancreatin 1:** A 1-ounce bottle of pancreatin (Parke, Davis & Co.); an old preparation.

**Trypsin 1:** A 1-ounce bottle of trypsin (Merck); purchased in September, 1912.

**Trypsin 2:** A 200-gm. bottle of trypsin sicc. (Greubler); imported about March, 1912.

**Trypsin 3:** A 50-gm. bottle of trypsin (Merck); purchased in August, 1913.

In every case the unopened bottle of enzym preparation was used. Portions were transferred to weighing bottles and dried for several days in desiccators until the loss in weight was slight. The bottles were then stoppered. The day before being used the enzym preparations were dried in a desiccator and portions weighed as needed.

In order to correct the digestion data for nitrogen introduced in the form of enzym, their nitrogen contents were determined. The results are summarized in Table VII. The methods used were similar to those employed throughout the work.

TABLE VII. —Quantity of N/5 nitrogen per gram of dry enzym preparation

Preparation	Total nitrogen.	Ammonia nitrogen	Amino nitrogen
	C. c.	C. c.	C. c.
Pepsin 1.....	51.1	.....	.....
Pancreatin 1. . . . .	40.9	.....	.....
Trypsin 1.....	47.3	None.	.....
Trypsin 2.....	70.1	62.5	2.0
Trypsin 3. . . . .	47.0	1.0	22.8

All of the digestion experiments were begun with freshly hashed beef or immature veal, except experiments 5 to 8, in which powdered meats beef sample 1.1 and veal sample 1.1, were used. These were prepared as follows:

**Veal sample 1.1:** Seven kgm. of veal sample 1 were hashed and transferred to two 8-liter wide-mouth bottles. Seven liters of 50 per cent alcohol were added and the mixture well stirred. After 24 hours the 50 per cent alcohol was strained off through cheesecloth and replaced with an equal volume of 75 per cent and the next day with 95 per cent alcohol. This was followed by two treatments with 2 liters of absolute ether. The ether was removed by straining through cheesecloth and squeezing

the material, after which most of the ether was removed by exposing the veal to the air in large crystallizing dishes. The veal was then heated in the hot-water oven at 85° C. (flame out) for two hours, and bottled.

Beef sample 1.1: Fourteen hundred grams of beef sample 1 were treated with alcohol and ether in exactly the same way as veal sample 1.1, using 1,400 c. c. of alcohol, etc.

When portions of these powdered-meat preparations were weighed for digestions, portions were also weighed for moisture determinations, so that the final weights were based on the dry material. Total nitrogen per gram of beef sample 1.1, 57.2 c. c. *N*/5 nitrogen; per gram of veal sample 1.1, 57.8 c. c. *N*/5 nitrogen. Other analytic data are given in Table V.

#### FIRST METHOD: WEIGHING THE UNDIGESTED MEAT RESIDUES

Portions of 5 gm. each of the raw hashed beef and veal were weighed into 200 c. c. Erlenmeyer flasks. After adding 40 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for 1 hour. They were then cooled, weighed, and the evaporated water replaced. To each flask 50 c. c. of 0.4 per cent hydrochloric acid were added, followed shortly afterwards by the addition of 10 c. c. of the pepsin solution. Three flasks containing beef and three containing veal were generally used in a single experiment (see Table VIII). In the controls on the acid 10 c. c. of water instead of the pepsin solution were added.

The digestion was considered to have begun when the pepsin was added. During the digestion period the flasks were rotated occasionally, so as to mix the contents. When the digestion period had ended, the filtration of the residue, consisting of undigested meat, fat, etc., was begun. For this purpose loose-textured filter papers (Schleicher & Schull's No. 589, white band, 15 cm.) were used. These papers, contained in weighing bottles, had previously been dried for several hours at 95° C. in the hot-water oven until the change in weight after a second drying was slight. Drying such papers to absolutely constant weight was as difficult as drying meat to constant weight without decomposition or oxidation.

It is at this point that the worker loses control over the method. When filtration was rapid, which sometimes happened, the separation of undigested meat from the pepsin-hydrochloric-acid solution ended the digestion period quite sharply, so far as the residue was concerned. But, as was generally the case, filtration was slow because the residue was gelatinous and clogged the filter, and it was not possible to end the digestion period shortly after filtration was begun because digestion continued as long as the pepsin-hydrochloric-acid solution was in contact with undigested meat. Fortunately, the digestive process becomes slow as the meat approaches complete digestion, so that the error from this source probably amounts to less than 10 per cent of the correct result.

When filtration was complete or nearly so, the residues were washed with water, transferred with the paper to the corresponding weighing bottles, and dried to approximately constant weight at 95° C. in the hot-water oven. From the data for moisture, the original 5-gm. portions of fresh meat were calculated to the dry weight. The weight of the dry, undigested residue divided by the corresponding weight of dry meat gave the percentage of beef or veal present as undigested residue (see "Percentages of meat digested," p. 700).

ACID PROTEINATE.—The value of the determination of acid proteinate in digestion mixtures has been pointed out by Gies (Hawk and Gies, 1902). The first step in the

digestion of a protein by pepsin-hydrochloric acid solution is the combination of the protein and the acid to form a class of substances known as acid proteinates. These are soluble in dilute acids and alkalies, but are insoluble in water.

The filtrates obtained at the end of the digestion period contained (1) the acid proteinates and (2) the next cleavage products of the acid proteinate, the proteoses and peptones. A measured amount of filtrate, generally between 50 and 80 c. c., taken before the washing of the residue was begun, was nearly neutralized with  $N/5$  sodium hydroxid. The exact amount added varied in the different experiments; calculated to 100 c. c. of filtrate it varied around 21 c. c. The 100 c. c. of 0.2 per cent hydrochloric acid in which the digestions were made were equivalent to 28 c. c. of approximately  $N/5$  sodium hydroxid. The addition of alkali was stopped when a flocculent precipitate of acid proteinate was thrown down. The mixture was then rapidly brought to a boil and filtered on a weighed paper. This was dried along with the undigested residues, and the results calculated in the same way.

The difficulties involved in promptly checking the action of the pepsin at the end of the digestion period were very apparent to Grindley, Mojonner, and Porter (1907, p. 68), who after many trials found that the addition of formaldehyde solution to a digestion mixture brought the digestion to a close. Differences in length of time required for filtration will not then involve the error previously mentioned. This method, however, is not the only one. By using small amounts of pepsin the digestion period may be made long; and then it makes little difference whether a particular mixture requires a few more or a few less hours to filter completely. An objection to this procedure is that the acid alone in the control may digest as much as the acid plus the small amount of pepsin, and the action of the pepsin under such conditions can not be measured with certainty. Further, the amount of pepsin must not be large enough to permit the digestive processes to go to completion, for the undigested residue then obtained represents material not digestible under the conditions, and no information is obtained regarding the rate at which digestion took place. If allowed time enough, both a fast horse and a slow horse will be found at the same place at the end of a race. In experiment 13, Table VIII, the undigested residues obtained after long digestion with fairly large amounts of pepsin represented the amount of meat constituents not digestible by the pepsin-hydrochloric-acid solution.

No information as to whether the beef or the veal digested faster could be obtained from such data. That the residues in this experiment were almost certainly fat is indicated by the results of Table IX, with which experiment 13 is comparable because the concentration of pepsin was the same in both—i. e., 10 mgm. to 100 c. c. of 0.2 per cent hydrochloric acid. Under these conditions practically all of the nitrogen in the beef and veal went into solution in 24 hours, leaving the fat, which is not digested by pepsin-hydrochloric-acid solution. Fat determinations were not made. According to Fish (1911, p. 132), beef contains more fat than ordinary veal. This is probably still more true of immature veal. The larger residues from beef in experiment 13 are in accord with the data of Fish.

TABLE VIII.—Comparative digestibility of mature beef and immature veal in pepsin-hydrochloric-acid solution

## BEEF AND VEAL SAMPLES I

Experiment No.	Digestion period.	Filtrate neutralized after—	Pepsin i.	Percentage of beef present as—		Percentage of veal present as—		Temperature.
				Undigested residue.	Acid proteinate.	Undigested residue.	Acid proteinate.	
			Mgm.	Per cent.	Per cent.	Per cent.	Per cent.	°C.
1....	8 days.....	4 hours.....	.....	79	4	74	5	Room.
			.....	79	8	73	5	
			0.01	64	16	63	9	
			.....	63	12	60	10	
2....	8 days.....	4 hours.....	.....	22	31	29	21	Do.
			.....	25	29	26	22	
			.....	84	4	85	4	
			10.0	43	(a)	52	5	
3....	1½ hours.....	½ hour.....	10.0	43	11	54	6	40
			.....	66	22	45	12	
			10.0	30	14	34	7	
			10.0	24	16	27	8	
4 <sup>b</sup> ....	3 hours.....	½ hour.....	.....	.....	.....	.....	.....	40
			.....	.....	.....	.....	.....	
			.....	.....	.....	.....	.....	
			.....	.....	.....	.....	.....	

## BEEF AND VEAL SAMPLES I, I

5 <sup>c</sup> ....	46 days.....	24 hours.....	.....	12	11	20	16	Room.
			.....	11	37	37	4	
			.....	8	23	43	34	
			.....	82	11	78	10	
6....	10 days.....	10 hours.....	.....	15	38	42	34	Do.
			.....	17	38	34	35	
			.....	88	10	94	6	
			10.0	11	25	33	21	
7....	4 hours.....	1 hour.....	10.0	11	26	33	21	Do.
			.....	87	.....	85	.....	
			10.0	25	.....	27	.....	
			10.0	25	.....	28	.....	
8....	.....	3 hours.....	.....	.....	.....	.....	.....	40
			.....	.....	.....	.....	.....	
			.....	.....	.....	.....	.....	
			.....	.....	.....	.....	.....	

## BEEF AND VEAL SAMPLES 2

9....	4 hours..	½ hour.....	.....	84	6	79	4	40
			10.0	27	7	21	7	
			10.0	30	7	21	6	
			.....	78	.....	73	.....	
10....	.....	4 hours.....	10.0	42	.....	32	.....	40
			10.0	44	.....	29	.....	
			.....	76	11	77	3	
			10.0	36	9	25	6	
11....	4 hours.....	½ hour.....	10.0	44	9	28	6	40
			.....	80	.....	77	.....	
			10.0	53	.....	32	.....	
			10.0	50	.....	33	.....	
12....	.....	4 hours.....	.....	74	14	73	9	40
			10.0	20	0	15	0	
			10.0	15	0	12	0	
			10.0	20	0	12	0	
13....	23 days.....	4 hours.....	10.0	23	0	11	0	Room.
			10.0	19	0	.....	.....	
			.....	.....	.....	.....	.....	
			.....	.....	.....	.....	.....	

<sup>a</sup> Determination lost.<sup>b</sup> The flasks containing the 5-gm. portions of hashed beef and veal were kept in cold storage at 2° C. for three weeks, during which time autolysis went on. This probably accounts for the small residues in the blanks, which contained hydrochloric acid but no pepsin. While in cold storage the flasks contained nothing but the meat.<sup>c</sup> Experiment 5 is to be rejected. The continued action of molds during the digestion period invalidated the results.

A second method of checking the action of the pepsin-hydrochloric-acid solution used in experiments 8, 10, and 12 involved nothing more than the neutralization of the digestion mixture at the end of the desired time. Pepsin digests in the presence of free acid; it does not act in neutral solutions with any appreciable speed. Thus in experiment 10, and in experiment 12, which was a repetition of experiment 10, exactly four hours after the digestion was begun by adding the pepsin solution to 5-gm. portions of meat suspended in 100 c. c. portions of 0.2 per cent hydrochloric acid, the entire mixture was neutralized by the addition of 21 to 25 c. c. of *N*/5 sodium hydroxid. This checked the peptic action at once, but also precipitated the acid proteinate. The mixture was then quickly brought to a boil, after which filtration, whether fast or slow, may be continued at the convenience of the worker. Obviously the residue in this case does not give as detailed information as that obtained by filtration of undigested residue and precipitation of acid proteinate in the filtrate. In experiments 10 and 12 the veal digested a little faster than the beef.

In experiments 5 to 8, practically fat-free beef and veal, prepared as described on page 685, were used. The object was to eliminate the error due to the fat, which, when present, is weighed with the undigested protein. One-gm. portions of the dry powders were used instead of the 5-gm. portions of fresh meat. Otherwise the procedure was the same as in the other experiments, except that, in so far as the proteins present had already been coagulated by exposure to alcohol, ether, and a temperature of 85° C., heating the mixture of meat powder and water in a boiling-water bath was omitted. The results in experiment 5 were invalidated by molds. In experiments 6 to 8 the results indicate a slightly more rapid digestion of beef sample 1.1.

The most interesting results in Table VIII are those of experiments 1, 2, and 6. In experiment 1 so minute a quantity of pepsin as 0.01 mgm. in 100 c. c. of 0.2 per cent hydrochloric acid exerted an equally distinct digestive action on both the beef and veal. With 0.1 mgm. of pepsin the digestion was unmistakable, indicating that in these particular cases the immature veal was as susceptible to the action of minute amounts of pepsin as the mature beef. To ascertain whether this was true or not was the object of experiments 1 and 2.

It will be noticed that in the experiments summarized in the table the amounts of pepsin used varied from 0.01 mgm. to 1,000 times this amount—i. e., 10.0 mgm. A wide range of enzym concentration in such work is not only desirable but almost necessary. What is true at one concentration of enzyme may not be true at another very different one. Thus, Berg and Gies (1907) found that in acetic acid fibrin would digest very slowly when the amount of pepsin present was comparatively small, but in the presence of large amounts of this enzyme digestion proceeded with a wholly unexpected speed.

A comparison of the results for beef in Table VIII with some of the data obtained by Grindley, Mojonnier, and Porter (1907, p. 66) in their artificial-digestion experiments can not very well be made. These investigators used 250 mgm. of pepsin per 100 c. c. of 0.33 per cent hydrochloric acid. The kind of pepsin preparation used was not stated, but, assuming it to be the usual 1 to 3,000 product, their digestion mixtures contained 25 times as much pepsin as the strongest digestion mixtures mentioned in Tables VIII or IX. Their conditions of comparatively high pepsin and high acid concentration probably were not favorable for the detection of small differences in digestibility, although these conditions may have been desirable for other reasons.

Perhaps the only work with which the data of Table VIII can be compared are the recent results obtained by Fish (1914) on the comparative digestibility of beef, market veal, and immature veal. In the absence of a statement pertaining to the treatment of the meats, the inference may perhaps be drawn that the digestion experiments were made on raw meats. Otherwise, the general method and conditions of Fish's digestion experiments were similar to those in experiments 1 to 13. Samples from 22 immature



veal calves were compared with an almost equal number of samples of market veal and beef, using "3.35 milligrams of scale pepsin" in 100 c. c. of 0.2 per cent hydrochloric acid. Fish (p. 52-53) concludes this part of the work with the following statement:

The results show that, as regards the averages, the differences in the digestibility of the tissues of bob veal and market veal are so slight as to be negligible; but such as they are, they are slightly in favor of the bob veal as a whole. The differences between the beef and veal is [sic] more noticeable, but the apparent greater digestibility of the veal may be due in part to the fact that as a rule there is a slightly smaller percentage of water present in the beef as well as a somewhat greater amount of connective tissue. As the greatest difference shown by the averages is but 3 per cent under the conditions of the experiments, it would indicate no serious difficulties in the digestibility of any of the material.

A redeeming feature of the method used in experiments 1 to 13 is its simplicity, both in the technic used and the equipment required. That the results obtained are substantially correct is indicated by the fact that repetitions of the measurements, using different methods, involving different errors, yielded similar results.

#### SECOND METHOD: MEASURING THE RATE OF FORMATION OF PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN

Into each of two 2-liter Jena Erlenmeyer flasks 100 gm. of freshly hashed beef were weighed to the 0.1 gm. Similar portions of veal were weighed into two similar flasks. After adding 750 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for one hour. They were then cooled, weighed, and the evaporated water replaced. The stoppered flasks remained in cold storage overnight. Two of these, one of beef and one of veal, were used for the determination of extractive nitrogen as already described on p. 673. The next morning the flask containing the beef and the flask containing the veal for the digestion experiment were quickly warmed to 40° C. The dry, powdered enzyme was then added, followed by 1 liter of 0.4 per cent hydrochloric acid or of 1 per cent sodium-carbonate solution. Water was then added to bring the final volume up to 2,000 c. c. In this way every digestion experiment was begun with 100 gm. of beef or veal, plus 2,000 c. c. of 0.2 per cent hydrochloric acid when pepsin was used (see Table IX), or 2,000 c. c. of 0.5 per cent sodium carbonate when trypsin was used (see Table X). During the course of the digestion the flasks were kept in a 40° C. water bath, except when they were removed to mix their contents or to take samples for analysis. The treatment of the digestion mixtures containing pepsin-hydrochloric-acid solution and those containing trypsin-sodium carbonate solution will be described separately.

**DIGESTION IN PEPSIN-HYDROCHLORIC-ACID SOLUTION.**—During the earlier part of the experiment the contents of the flasks were mixed about every 15 minutes. Later, when most of the meat had gone into solution, the mixing was done at longer intervals, but always the same for both flasks. In the experiments summarized in Table IX the rate of digestion was measured at the time intervals there indicated by removing 100 c. c. portions of supernatant digestion fluid and determining in this the amount of nitrogen present as acid proteinate, proteoses, and peptones. By difference the nitrogen in the undigested residue could be obtained. If, for example, it was desired to obtain data on veal for one hour's digestion, the veal mixture was well mixed 45 minutes after the digestion was begun and was allowed to remain in the water bath for 10 minutes, in order to allow meat particles to settle to the bottom of the flask. The flask was then removed from the bath, and with a calibrated 100 c. c. pipette 100 c. c. of the supernatant suspension was transferred to a 200 c. c. Erlenmeyer flask. Exactly 60 minutes after the digestion began, the action of the pepsin-hydrochloric-acid solution was stopped by nearly neutralizing the contents of the 200 c. c. Erlenmeyer flask by the addition of *N*/5 sodium hydroxid and bringing it to a boil by heating directly over a Bunsen burner. The flask containing the digestion mixture was

replaced in the bath. The quantities of  $N/5$  sodium hydroxid used varied from 18 to 29 c. c. The neutralization is satisfactory when a flocculent precipitate appears. In the same way 100 c. c. of the digestion fluid from the beef mixture were removed and neutralized 60 minutes after starting the beef digestion.

In this way portions of the digestion mixtures of beef and veal were removed for neutralization on the minute, at intervals of 1, 2, 4, 7, and 24 hours. Fifteen minutes before neutralization the flask contents were mixed and allowed to stand for 10 minutes. A 100 c. c. portion was then removed from the bulk of the digestion mixture 5 minutes before neutralization.

The precipitated acid proteinate was filtered, washed, and nitrogen was determined by the Kjeldahl method. The results obtained are given in Table IX under the heading "Quantity of  $N/5$  acid-proteinate nitrogen."

The filtrate was transferred to a Kjeldahl flask and the total nitrogen determined. This filtrate contained nitrogen derived from (1) the proteoses and peptones formed by the digestion of the meat, (2) the extractives present before digestion began, and (3) the pepsin. The figure for total nitrogen obtained on the filtrate is the sum of these three. The data recorded in Table IX under the heading "Quantity of  $N/5$  proteose and peptone nitrogen" are the figures actually obtained and corrected for the sum of the extractive and pepsin nitrogen. Thus, in experiment 14 the results obtained for one hour's digestion of beef sample 3 were, for the precipitated acid proteinate, 2.7 c. c. of  $N/5$  nitrogen; for the filtrate, 23.8 c. c. From this latter figure there was subtracted 8.0 c. c., this being the sum of the extractive nitrogen in that sample of beef at that time, and the nitrogen present in the pepsin added. The method of determining extractive nitrogen is described on page 673.

During the digestion the water contained in the meat is liberated and dilutes the digestion fluid to a slight extent. No correction for this was made, except in those particular cases where the correction is indicated.

The "theoretical maximum" for proteose and peptone nitrogen in 100 c. c. of digestion fluid was calculated in the following manner: The sum of the total nitrogen in 100 gm. of fresh meat plus the pepsin nitrogen was divided by the volume of the digestion fluid at complete digestion—i. e., 2,000 c. c. plus the volume of water in the 100 gm. of meat.

By the term "Age of meat, days," at the bottom of Table IX is meant the number of days the meat was in cold storage before being boiled. Thus, in experiment 21 beef sample 6 and veal sample 6 were hashed and boiled after 13 days in cold storage, and on the next day digestion was begun. These figures do not refer to the age of the calf when killed, this having been given in Table I.

It will be noticed that the theoretical maximum for proteose and peptone nitrogen is approximately 50 c. c. of  $N/5$  nitrogen in nearly all the experiments. In order to obtain the percentage of nitrogen present as proteoses and peptones at any time, it is only necessary to multiply the corresponding figure by 2. Thus, in experiment 19, at the end of seven hours approximately 82 per cent of the veal (41.0+48.0) had been transformed into proteoses and peptones. It is obvious that both the beef and the veal were digested with practically the same speed and that at the end of 24 hours the transformation into proteoses and peptones was complete.

For practical purposes the digestive process may here be regarded as taking place in two stages: (1) The transformation of the native meat proteins to acid proteinate by combination with the hydrochloric acid, and (2) the cleavage of the acid proteinate into the smaller molecules of proteoses and peptones.

The data in Table IX indicate that both processes took place with equal speed in the beef and veal.

The undigested residues weighed in experiment 13, Table VIII, probably contained very little nitrogen. The concentration of pepsin in experiments 9 to 13 was the same

as in the experiments in Table IX. By comparing the results of experiment 13 with those of experiment 14, for example, it will be apparent that the undigested residues in experiment 13 give an imperfect idea of the amount of indigestible protein present in beef and veal; according to the data of Table IX practically all of the nitrogen was in soluble form at the end of 24 hours.

The conditions of the experiments in Table IX were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.2 per cent hydrochloric acid plus 200 mgm. of pepsin 1. For nitrogen determinations 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE IX.—Rate of formation of proteoses and peptones in pepsin hydrochloric-acid solution

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 PROTEOSE AND PEPTONE NITROGEN

Digestion period.	Experiment No. —									
	14		17		19		21		23	
	Beef sample 3.	Veal sample 3.	Beef sample 5.	Veal sample 5.	Skim milk sample 1.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
<i>Hours.</i>										
1 .....	15.8	10.3	15.6	16.8	27.7	12.6	15.7	12.1	17.1	11.6
2 .....	24.7	20.5	26.8	26.6	37.2	23.4	23.4	19.3	27.9	18.5
4 .....	33.8	31.1	37.8	36.3	43.8	34.6	33.1	29.6	37.9	29.0
7 .....	41.8	39.5	45.3	41.4	46.2	41.0	41.0	37.8	44.2	36.7
24. ....	50.7	47.1	52.3	46.7	50.4	46.6	52.8	(a)	52.9	45.5
Theoretical maximum	51.8	48.3	53.1	48.0	54.8	48.0	53.8	43.8	54.2	47.3
Extractive nitrogen..	7.5	8.9	7.2	7.8	3.7	7.8	8.1	7.7	7.5	11.1
Pepsin nitrogen...	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 ACID PROTEINATE NITROGEN

<i>Hours.</i>										
1 .....	2.7	1.5	4.6	4.2	26.0	2.4	2.8	1.6	3.3	1.5
2 .....	3.9	2.8	4.8	4.9	17.3	4.5	4.7	3.2	5.2	3.7
4 .....	3.4	3.6	5.0	4.6	10.8	4.6	5.4	4.3	4.9	4.0
7 .....	3.3	4.3	3.8	5.1	8.2	5.6	5.2	5.9	4.1	4.2
24. ....	3.0	4.0	3.3	4.2	4.3	4.9	3.7	4.5	2.7	4.0
Age of meat, days.....	8	8	0	0	18	18	13	13	3	3

<sup>a</sup> Determination lost. Result obtained at 53 hours (47.1 c. c.) is probably incorrect, being larger than the theoretical maximum for that mixture.

The results of the experiments in Table IX can be plotted, and curves, of which the following are typical, obtained (fig. 1).

After several comparisons of veal with beef showed no appreciable differences between the two as regards their behavior in pepsin hydrochloric acid or in trypsin sodium carbonate, it was desirable to compare the veal with some other protein material in order to be certain that the method used would detect a difference in the

rate of digestion when such a difference existed. Accordingly, in experiment 19, veal sample 5 was compared with a sample of raw skim milk obtained in the fresh condition from the Dairy Division, Bureau of Animal Industry. Instead of 100 gm. of beef, 600 gm. of the skim milk were transferred to a 2-liter Erlenmeyer flask. The specific gravity of skim-milk sample 1 was 1.0352 at 26° C., and, hence, the volume of the 600 gm. was  $600/1.0352$ , or 579.2 c. c. This was regarded as if it were 100 gm. of beef plus 479 c. c. of water. To this amount, 316 c. c. of water were added, the milk being kept in a boiling-water bath for five minutes. It was kept in cold storage overnight with veal sample 5; the next morning it was treated in the usual way along with this sample. At the beginning of the digestion the volume of the skim-milk digestion mixture was 2,096 c. c., which is practically the volume of the meat mixtures—i. e., 2,000 c. c. plus the volume of 100 gm. of meat, which lies between 75 and 100 c. c. A similar sample of skim milk in 0.2 per cent hydrochloric acid was used for the determination of extractive nitrogen. Skim-milk sample 1 con-

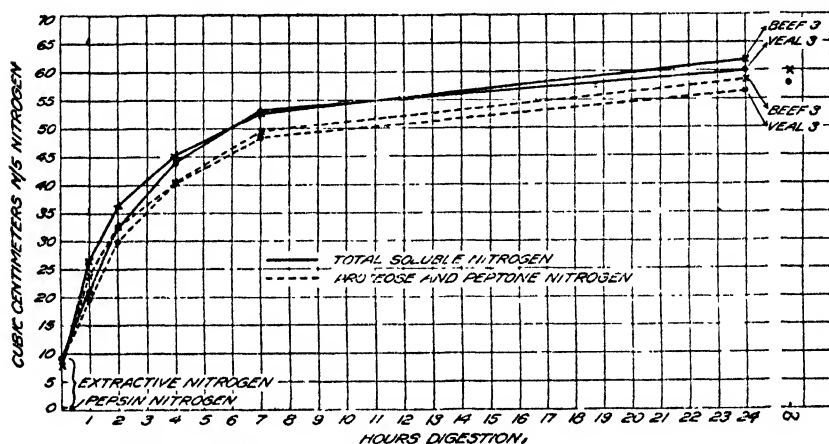


FIG 1—Experiment 14. Curve showing the quantity (in cubic centimeters) of  $N/5$  nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat; used 100 gm. of meat, 2,000 c. c. of 0.2 per cent hydrochloric acid, and 200 mgm. of pepsin 1.

tained 2.05 c. c. of  $N/5$  nitrogen per gram, or 0.574 per cent. The extractive nitrogen was 6.3 per cent of the total nitrogen.

In precipitating the undigested proteins and the acid proteinate by neutralization and heat, care was taken to test the filtrates with acid and alkali, in order to be certain that precipitable protein was not present in any of the filtrates. The complete precipitation, though troublesome, was not difficult. The precipitates, containing both undigested proteins and acid proteinate, were determined for nitrogen by the Kjeldahl method in the usual manner and the results recorded under the heading "Quantity (in cubic centimeters) of  $N/5$  acid proteinate nitrogen." The figures for proteose and peptone nitrogen obtained from the filtrates indicate that this transformation was more rapid in the skim milk than in the veal. This is, of course, easily accounted for by the fact that the skim-milk proteins were in solution or suspension at the beginning of the digestion, while the veal particles took time to go into solution.

**DIGESTION IN TRYPSIN SODIUM CARBONATE SOLUTION.**—In general, these experiments were carried out in exactly the same way as the digestions in pepsin hydrochloric acid solution. Dry, powdered trypsin preparations were used. Portions of these were weighed and transferred to the digestion mixtures in the same way as the pepsin. Instead of 1 liter of 0.4 per cent hydrochloric acid, the same volume of 1 per cent sodium carbonate was added. The digestions in experiments 15 to 34 (Tables X and XI)

were all made in 0.5 per cent sodium carbonate. Although trypsin 1 and trypsin 3 had the same total nitrogen contents (see Table VII), trypsin 1 was the more active preparation. This is evident from the fact that in experiments 18, 20, and 22 (Table X) digestion had proceeded as far in seven hours as in experiments 32 and 34 at the end of six hours, although in the latter experiments twice the weight of trypsin was used.

The 100 c. c. portions of digestion fluid were neutralized with 24.5 c. c. of 2  $N \frac{2}{5}$  sulphuric acid, the exact strength of which was  $N \frac{2}{5} \times 0.98$ . This was sufficient to neutralize the sodium carbonate present and leave about 0.5 c. c. of the acid in excess, preventing the escape of ammonia when the mixture was brought to a boil. The filtration and determination of total nitrogen in the precipitated alkali proteinate and in the filtrate were carried out as described in the acid digestions.

It is to be noted that, while small amounts of pepsin in hydrochloric acid will rapidly digest meat proteins to the proteose and peptone stage but no further, trypsin, although much slower in its action, will further split the meat proteins into amino acids. This is the reason for the data under "Quantity of  $N \frac{2}{5}$  proteose, peptone, and amino-acid nitrogen" in Tables X and XI. The statement of results in Table X is, in general, similar to that in Table IX.

The conditions of experiments 15 to 24 were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium-carbonate solution plus 2.000 gm. of trypsin 1; except experiment 15, in which 2,000 gm. of pancreatin 1 was used. For nitrogen determinations, 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE X.—Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution

		Experiment No.											
Digestion period.		15		16		18		20		22		24	
		Beef sample 4.	Veal sample 4.	Beef sample 4.	Veal sample 4.	Beef sample 5.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
Hours.													
1.....	5.0	6.5	9.3	7.7	11.4	9.3	9.2	8.4	7.7	6.2	7.8	8.1	
2.....	10.7	11.7	16.9	15.4	20.7	16.6	18.5	16.0	14.5	13.4	13.8	14.0	
4.....	16.5	17.9	<sup>a</sup> 28.3	<sup>a</sup> 28.1	29.8	25.6	29.1	24.6	23.0	21.1	20.3	19.2	
7.....	22.2	23.5	<sup>a</sup> 34.1	<sup>a</sup> 34.7	38.7	33.3	37.4	31.5	30.2	26.2	26.1	22.9	
24.....	33.7	34.1	42.9	43.6	47.6	42.2	46.7	40.6	43.7	36.8	36.9	28.5	
Theoretical maximum	52.7	46.1	53.2	46.6	53.4	48.3	54.1	44.1	54.1	44.1	50.1	32.5	
Extractive nitrogen...	7.2	8.4	7.2	8.4	7.2	7.8	8.1	7.7	8.1	7.7	11.9	26.2	
Trypsin nitrogen.....	4.1	4.1	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	

QUANTITY (IN CUBIC CENTIMETERS) OF  $N \frac{2}{5}$  ALKALI-PROTEINATE NITROGEN

1.....	3.7	2.9	3.7	2.0	3.3	2.9	4.0	2.6	3.0	2.3	3.1	3.9
2.....	6.8	4.2	6.8	5.3	4.2	4.4	4.8	4.0	4.0	6.5	4.7	3.0
4.....	7.6	5.1	<sup>a</sup> 6.2	<sup>a</sup> 5.3	4.2	5.7	5.0	5.0	4.3	9.8	5.0	3.3
7.....	8.6	6.9	<sup>a</sup> 5.7	<sup>a</sup> 8.2	4.4	7.6	5.5	5.8	4.8	10.6	4.9	2.0
24.....	6.9	8.3	3.3	4.2	4.3	4.9	3.1	5.1	3.0	6.7	3.6	1.6
Trypsin nitrogen.....	.....	.....	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Age of meat, days ...	1	1	9	9	6	7	2	2	21	21	33	33

<sup>a</sup> Results are for five and eight instead of four and seven hours.

In experiments 15 to 24, because of the comparatively large weight of trypsin used, it was desirable to ascertain how much of the trypsin nitrogen appeared in the neutralized digestion filtrate and in the precipitate of alkali proteinate, in order that both may be corrected by the amounts found. Accordingly, two portions of trypsin 1, each weighing 100 mgm., were dissolved in 100 c. c. of 0.5 per cent sodium carbonate and precipitated with 48 c. c. of *N*/5 sulphuric acid, as in the digestion experiments. The mixtures were heated to a boil and filtered. The total nitrogen (*N*/5) in the filtrates was 4.25 and 4.40 c. c.; in the precipitates, 0.47 and 0.70 c. c. The averages of these are recorded in Table X, and both were used as corrections, as already described on page 691. For trypsins 2 and 3 the term "trypsin nitrogen" in Table XI means the total nitrogen in the trypsin present in the 100 c. c. of digestion fluid. Trypsin 2 contained approximately 90 per cent of its nitrogen as ammonia, and consequently the amount precipitated with the alkali proteinate was disregarded. The results for alkali proteinate in experiments 31 to 34 with trypsin 3 showed that the correction for alkali proteinate derived from the trypsin must have been similar to that in trypsin 1, and the determination of this correction was omitted.

In experiment 18, for example, 100 c. c. of veal sample 5 digestion fluid were neutralized exactly four hours after the digestion began, and the mixture was brought to a boil and filtered. The filtrate contained 37.7 c. c. of *N*/5 nitrogen, of which 4.3 c. c. were derived from the trypsin present and 7.8 c. c. from the extractives present before the digestion was begun; and the figure recorded, 25.6 c. c., is the amount of proteose, peptone, and amino-acid nitrogen actually formed by the digestive process. The precipitated alkali proteinate contained 6.3 c. c. of *N*/5 nitrogen, of which 0.6 c. c. was derived from the trypsin. The corrected figure, 5.7 c. c., is recorded in Table X.

The results with trypsin are practically the same as those with pepsin. They indicate that both the beef and the veal digested with practically the same speed. The presence of only small amounts of alkali proteinate through the experiments indicates that just as soon as the beef or the veal goes into solution as alkali proteinate this is promptly split into the simpler molecules of proteoses, etc.—i. e., the equality in speed of digestion pertains both to the first and to the later stages in the digestive process for both beef and veal. At no time was there any indication that either the beef or the veal contained any nitrogenous substances resistant to the action of the trypsin. In experiments 16 to 24, Table X, approximately 90 per cent of the veal had gone into solution at the end of 24 hours, with similar results for the beef.

In experiments 26 to 34, Table XI, the rate of digestion was measured by both the second and third methods. The comparisons between veal sample 9 and skim-milk sample 2 in experiments 27 and 28 were made for the purpose of ascertaining whether the method used would detect a difference in rate of digestion when such a difference was large. Experiment 28 was a repetition of experiment 27. On account of the comparatively vigorous action of pepsin-hydrochloric-acid solution veal sample 5 in experiment 19 very soon "caught up" with skim-milk sample 1; but in experiments 27 and 28 the striking difference between the rate of digestion of skim-milk sample 2 and veal sample 9 was brought out by the less vigorous cleavage of the trypsin-sodium-carbonate solution. The treatment of skim-milk sample 2 was similar to that of skim-milk sample 1. Skim-milk sample 2 was obtained by skimming, with the aid of a siphon, a sample of ordinary pasteurized milk obtained from a dealer. One gm. of skim-milk sample 2 contained 1.88 c. c. of *N*/5 total nitrogen, or 0.529 per cent. The extractive nitrogen in experiments 27 and 28 was 11.6 and 14.2 per cent, respectively, of the total. The specific gravity was 1.0334 at 26° C. Six hundred gm. of skim-milk sample 2 were weighed into a 2-liter Erlenmeyer flask. The calculated volume of the skim milk was 580.4 c. c. To this 316.4

gm. of water were added and the flask kept in a boiling-water bath for 15 minutes. The temperature inside the flask was  $89^{\circ}\text{C}$ . The evaporated weight of water was replaced. The heated skim milk was kept overnight in cold storage and digested the next morning with veal sample 9, after the addition of 2,000 gms. of trypsin 2, 1 liter of 1 per cent sodium carbonate, and 200 c. c. of water; total volume, 2,098 c. c. The neutralization of the 100 c. c. portions of digestion fluid were made, as usual, with 24.5 c. c. of  $N/0.4$  sulphuric acid, followed by heating to a boil. Extractive nitrogen was determined in a similar portion of skim-milk sample 2 in 0.5 per cent sodium carbonate; 27.5 and 29 c. c. of  $N/0.4$  sulphuric acid were used for the pre-

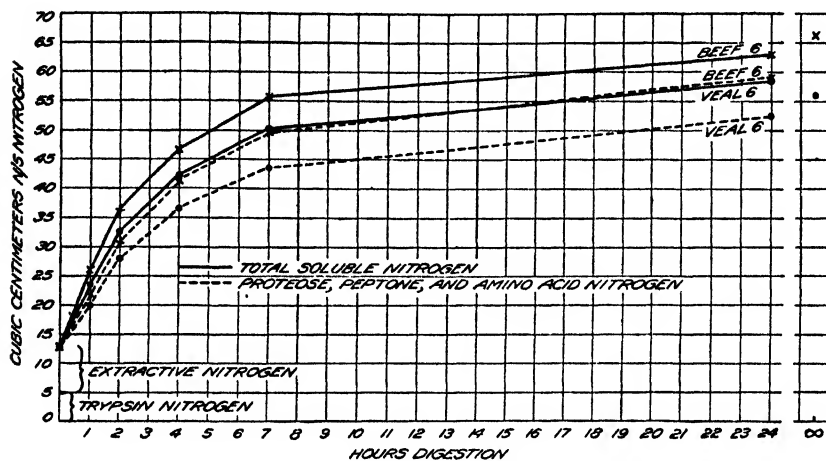


FIG. 2.—Experiment 20. Curve showing the quantity (in cubic centimeters) of  $N/5$  nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, used 100 gm. of meat, 2,000 c. c. of 0.5 per cent sodium carbonate, and 2,000 gm. of trypsin 1.

precipitation of 30 gms. of skim-milk sample 2 contained in 100 c. c. of 0.5 per cent sodium carbonate.

In the two following diagrams (figs. 2 and 3) the data of experiments 20 and 28 are graphically represented.

### THIRD METHOD: MEASURING THE RATE OF LIBERATION OF FREE AMINO GROUPS

These determinations were made on the same digestion mixtures used in experiments 26 to 34. Portions of 100 c. c. of the supernatant digestion fluid were removed for the determination of the nitrogen present as alkali proteinate, proteoses, peptones, etc., as already described. In addition, 10 c. c. portions of the digestion fluid were transferred to the Van Slyke amino-nitrogen apparatus, and free amino nitrogen was determined by the method already described on p. 680.

In this method, as in the previous ones, particular care was taken to check the action of the trypsin on the minute. The digestion mixtures in the  $40^{\circ}\text{C}$ . water bath were mixed 15 minutes before the time intended for the determination. The undigested meat particles were allowed to settle for 10 minutes. During this time the amino-nitrogen apparatus was made ready for the determination. Two or three minutes before the digestion period was to be brought to a close, 10 c. c. of the supernatant digestion fluid were transferred to the apparatus, and exactly at the expiration of the digestion period the digestion fluid was allowed to enter the reaction chamber of the apparatus. This brought the digestion to a close.

The results obtained are summarized in Table XII. Amino nitrogen in the extractives was determined in portions of the same filtrates that were used for total extractive-

nitrogen determinations (see p. 696). The results for amino nitrogen in trypsin 2 were obtained by introducing a solution of the enzyme in 0.5 per cent sodium carbonate directly into the amino-nitrogen apparatus. The ammonia nitrogen present in this preparation reacted completely with nitrous acid in the 20 minutes' reaction period used uniformly in the determinations. The small amount of ammonia nitrogen present in trypsin 3 permitted the determination of amino nitrogen in the residue obtained after ammonia removal and the use of this figure as the correction (see Table V II).

The conditions of experiments 26 to 34 were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium-

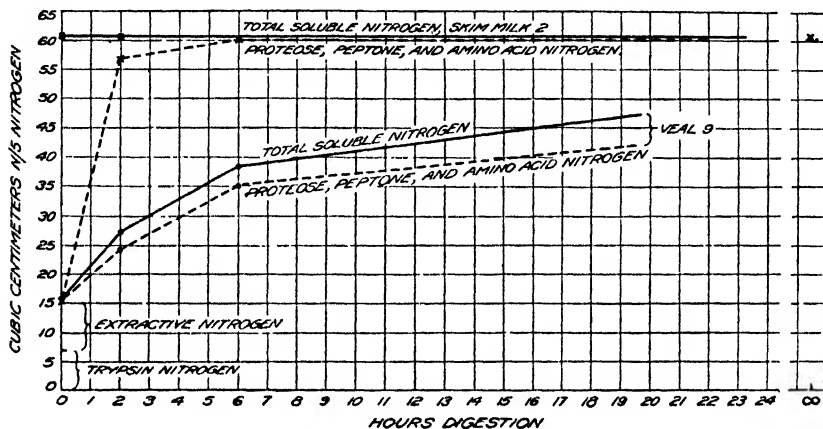


FIG. 3.—Experiment 28. Curve showing the quantity (in cubic centimeter) of  $N/5$  nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat or 30 gm. of skim milk; used 100 gm. of veal sample 9 or 600 gm. of skim-milk sample 2, 2,000 c. c. of 0.5 per cent sodium carbonate, and 2,000 gm. of trypsin 2.

carbonate solution plus the amount of trypsin indicated in Table XII. For nitrogen determinations, 100 c. c. digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE XI.—Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution

		QUANTITY (IN CUBIC CENTIMETERS) OF $N/5$ PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN															
		Experiment No. —															
Digestion period.		26 <sup>a</sup>		25 <sup>b</sup>		27		28		30		31		32		33	
		Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
Hours.																	
2...	...	22.2	18.2	17.0	18.2	44.3	9.3	41.8	10.4	14.7	13.7	9.7	11.4	18.4	18.4	21.5	19.7
6...	...	32.8	26.8	25.3	25.2	46.2	19.9	45.2	20.5	27.0	27.2	19.0	21.5	31.7	33.8	34.7	34.2
Theoretical maximum		52.4	41.9	50.5	41.8	47.1	45.3	45.6	44.5	49.3	41.3	49.1	40.1	51.2	45.6	51.3	45.1
Extractive nitrogen...		8.3	8.6	10.0	8.6	6.6	8.1	8.1	8.9	7.7	9.1	8.2	10.7	7.5	8.6	7.3	8.2
Trypsin nitrogen....		3.5	3.5	7.0	7.0	7.0	7.0	7.0	7.0	14.0	14.0	4.7	4.7	9.4	9.4	9.4	9.4

<sup>a</sup> Results obtained at end of 26 and 168 hours, instead of 2 and 6 hours.

<sup>b</sup> Results obtained at end of 7 and 24 hours, instead of 2 and 6 hours.



TABLE XI.—Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution—Continued

QUANTITY (IN CUBIC CENTIMETERS) OF  $N/5$  ALKALI PROTEINATE NITROGEN

Digestion period.	Experiment No. —															
	26		25		27		28		30		31		32		34	
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
Hours.																
2.....	5.9	3.7	6.2	5.1	2.9	3.0	3.6	3.0	3.2	3.1	2.8	3.5	3.7	4.9	4.8	5.1
6.....	5.0	7.0	5.2	6.5	.9	3.6	.0	5.1	3.9	3.9	4.5	5.0	3.7	3.8	2.3	3.5
Age of meat ...days	8	8	31	31	..	6	..	21	19	19	28	28	19	19	8	8
Trypsin used, gm.	1	1	2	2	2	2	2	2	2	4	2	2	4	4	4	4
Trypsin No.	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3

It is obvious that the amino nitrogen contained in the digestion fluid and actually determined was the sum of the amino nitrogen derived from (1) the trypsin; (2) the nitrogenous extractives, both of which were present before digestion began; and (3) the amino groups unlinked by the cleavage of the more complex proteoses into the simpler peptones and polypeptids. This is brought about by the action of the trypsin-sodium-carbonate solution during the digestion process. The results actually obtained in the determinations were diminished by the sum of 1 and 2, so that the figures in Table XII correspond to 3, or the amino nitrogen actually formed by the digestion. The minus quantities obtained in this way in some of the experiments for the 15-minute digestion period are probably due to the fact that the errors in determining the small amounts of amino nitrogen in 1 and 2 are large when compared with the small amount formed during 15 minutes' digestion.

## DISCUSSION OF THE DIGESTION EXPERIMENTS

**THEORETICAL MAXIMUM.**—If the digestion of the meat by trypsin could be brought to completion, the meat proteins would be split into simple amino acids. Such a complete cleavage of protein by a trypsin sodium-carbonate solution seldom, if ever, occurs. One reason is that the action of the trypsin becomes slower and slower the nearer the digestion process approaches completion. But by boiling the meat with hydrochloric acid, as already described (p. 678), the proteins and other nitrogenous substances are completely hydrolyzed, or 100 per cent digested. The data in Table XII, under the heading "Theoretical maximum," were obtained from Table V. The total amino nitrogen obtained from hydrochloric-acid hydrolysis minus the amino nitrogen in the extractives gave the figures recorded in Table XII. A slight error was here involved; the correction should have been the amino nitrogen in the extractives after acid hydrolysis, not before. For the present purposes this error is regarded as entirely negligible.

TABLE XII.—Rate of liberation of free-amino groups in trypsin-sodium-carbonate digestion mixtures

Digestion period	Quantity (in milligrams) of amino nitrogen in 10 c c of digestion fluid, equivalent to approximately 5 gm of meat in experiment No. —											
	26		25		27		28		30		31	
	Beef sample 8	Veal sample 8	Beef sample 8	Veal sample 8	Skim-milk sample 2	Veal sample 9	Skim-milk sample 2	Veal sample 9	Beef sample 10	Veal sample 10	Beef sample 11	Veal sample 12
14 hour.....	0.09	(b)	0.08	—0.41	0.56	0.01	0.51	—0.39	0.00	0.16	0.37	0.30
1 hour.....	0.20	c 25										
3 hours.....	0.64	0.43	0.72	0.67	1.93	0.20	1.92	0.73	1.42	1.39	2.76	0.89
6 hours.....	0.79	0.68	1.43	1.26	2.60	1.33	2.33	1.46	2.32	2.52	4.16	2.89
12 hours.....			1.94	1.82	2.67	1.73	2.71	1.93	3.44	2.82	5.47	4.45
24 hours.....			0.274	0.279	3.36	2.52	0.293	0.394	3.71	3.02	7.81	5.82
48 hours.....	1.30	1.19	2.95	3.68	0.381	3.95	0.394	0.539	5.39	5.52	6.44	7.93
72 hours.....	2.13	1.74	2.09	3.38	4.63	4.92	0.382	0.514	6.11	6.75	10.59	8.93
96 hours.....	0.235	0.01	2.51	4.33					6.83	7.40	13.07	11.14
120 hours.....			2.63	3.04	5.09				8.66	7.74	13.29	11.45
144 hours.....												
12 days.....												
Theoretical maximum.....	11.98	10.22	11.08	10.22	(c)	(c)	(c)	12.05	10.42	11.97	12.39	0.89
Amino nitrogen in extractives.....	.66	.46	.68	.46	1.11	.27	1.11	.27	.51	.53	.59	.47
Amino nitrogen in trypsin.....	.85	.85	1.71	1.71	1.71	1.71	1.71	1.71	3.42	3.42	1.28	1.28
Age of meat.....	8	8	31	31	6	6	21	19	19	2.8	19	8
Trypsin used.....	1	1	2	2	2	2	2	2	4	2	4	4
Trypsin No.....	2	2	2	2	2	2	2	2	2	3	3	3

a Results obtained one hour later than the time indicated, i. e., in experiment 26, the results were obtained for four and seven hours' digestion, etc.

b Determination lost.

c Low results obtained were rejected (see p 680)

**PERCENTAGE OF MEAT DIGESTED.**—In 0.5 gm. of meat the theoretical maximum amino nitrogen varies between 10 and 12 mgm. In order to convert the figures for amino nitrogen in Table XII to the percentage of the total amino nitrogen, it is only necessary to multiply them by a factor easily obtained mentally, which factor varies from 10 to 8.5. Thus, in experiment 32, 10 c. c. of the beef sample 11 digestion fluid contained 4.16 mgm. of amino nitrogen at the end of six hours. At complete digestion 12.39 mgm. would have been present; therefore  $4.16 \div 12.39$  or 34 per cent of the total amino nitrogen present had been unlinked by the cleavage of polypeptids under the conditions of the experiment. The same figure may be obtained directly by multiplying in round numbers 4 by 8. A minute before, or after, this particular amino-nitrogen determination was begun, a 100 c. c. portion of the same digestion fluid had been neutralized by the addition of 24.5 c. c. of *N*/5 sulphuric acid. This mixture was brought to a boil in the next few minutes, filtered, and total nitrogen was determined in the filtrate and the precipitate. The results were recorded in Table XI. This table shows that in the same experiment, No. 32, at the end of six hours' digestion of beef sample 11, approximately 60 per cent (i. e.,  $31.7 \div 51.2$ ) of the originally insoluble beef sample 11 nitrogenous substances had gone into solution as proteoses, peptones, and amino acids. These figures show how imperfect is the expression "Percentage of meat digested." The digestion process involves several chemical changes which take place at different rates. In general, the cleavage (by trypsin) of the larger molecules of alkali proteinate and proteose goes on at a comparatively rapid rate, the cleavage of the simpler peptone and polypeptid molecules at a slow rate. These facts are illustrated by the foregoing data of experiment 32. By the second method of measuring digestion it was shown (Table XI) that at the end of six hours' digestion 60 per cent of beef sample 11 had been transformed into proteoses, peptones, and amino acids; but by the third method of measuring digestion only one-third of the total amino nitrogen present had been unlinked (Table XII). The last two statements are correct; but it would not be entirely correct to say that according to the second method 60 per cent of beef sample 11 had digested at the end of six hours, or that 34 per cent of beef sample 11 under the same conditions had digested, using the third method of measuring digestion. A single figure can not describe several simultaneous processes in this case. The results in Tables XI and XII were obtained with the same digestion mixtures. The results in Table XII are expressed in milligrams of amino nitrogen obtained from 10 c. c. of digestion fluid, equivalent to approximately 0.5 gm. of meat.

**PRESERVATIVES NOT USED.**—In all the digestion experiments the flasks in which the meat was heated and later digested were partly sterilized by the heating in the boiling-water bath. During the diges-

tions in which the pepsin-hydrochloric-acid solution was used bacterial action was excluded from the digestion mixtures by the bactericidal action of the 0.2 per cent hydrochloric acid. During the digestions in which trypsin-sodium-carbonate solution was used bacterial action was not excluded, because any bacteria introduced into the digestion mixtures would not be destroyed by 0.5 per cent sodium carbonate. When the digestion period was short (Tables X and XI)—i. e., 24 hours or less—the possible error due to such recently introduced bacteria was negligible because the proteolytic action of the most vigorous proteolytic bacteria is very weak when compared with that of trypsin. When the digestion period was long enough (Table XII) the chemical changes brought about by the bacteria may have appreciably affected the results. No preservatives were used in any of the digestion experiments. This was regarded as an almost necessary condition in view of the fact that both the wholesomeness of immature veal and the influence of certain preservatives on digestion, health, etc., have been subjects of controversy. In the third method it was decided to carry on the digestions as aseptically as possible and to regard the results obtained in the first 48 hours as practically uninfluenced by bacteria. Generally after a few days putrefactive odors were noticed in the digestion mixtures. In so far as a very strong putrefactive odor can be caused by slight chemical changes in which small amounts of strongly odoriferous substances are produced, the amino determinations were made as late as 12 days after beginning the digestion in mixtures that were undoubtedly putrefying as judged by the odor. The practical necessity of a long digestion period in the third method, because of the slowness of amino-nitrogen liberation, together with the indeterminate effect of bacteria, is an objection to this method. The results of the first and second methods showed that under similar conditions mature beef and immature veal proteins were digested to the proteose and peptone stage with practically equal speed. However valuable such data may be they are not complete until the speed of the last transformation in the digestive process is measured for both. If the rate of liberation of amino groups in immature veal had been found to be slower than in mature beef, that fact would have constituted a good reason for the claim that immature veal digests with difficulty in the human digestive tract. The principal advantage of the third method as applied to digestion mixtures lies in the fact that it affords an easy, rapid method of measuring amino-nitrogen liberation, which can not easily be measured by other methods.

GRAPHIC REPRESENTATION OF RESULTS.—In figure 4 the results for amino nitrogen in experiment 32 are plotted. Most of the other curves obtained in this way were flatter because the rate of amino nitrogen liberation by trypsin 2 was slower. The curve for experiment 32 indicates that during the first 36 hours, approximately, the veal digested

a little more rapidly than the beef. After 48 hours the digestion mixture of veal sample 11 smelled putrid. In addition to the amino nitrogen liberated by the trypsin in this mixture non-amino nitrogen was transformed into amino nitrogen by the bacteria. This was indicated by the fact that after 48 hours' digestion amino nitrogen in veal sample 11 was higher than the amount originally present in the meat. During the bacterial and tryptic action which followed, practically all of the nitrogen was transformed to amino nitrogen. The mixture of beef sample 11 did not smell putrid in this experiment. In experiment 34, which was a repetition of experiment 32 except that beef and veal samples 12 were used, both mixtures from these samples had become putrid, and in both, as the data in Table XI show, the amino nitrogen

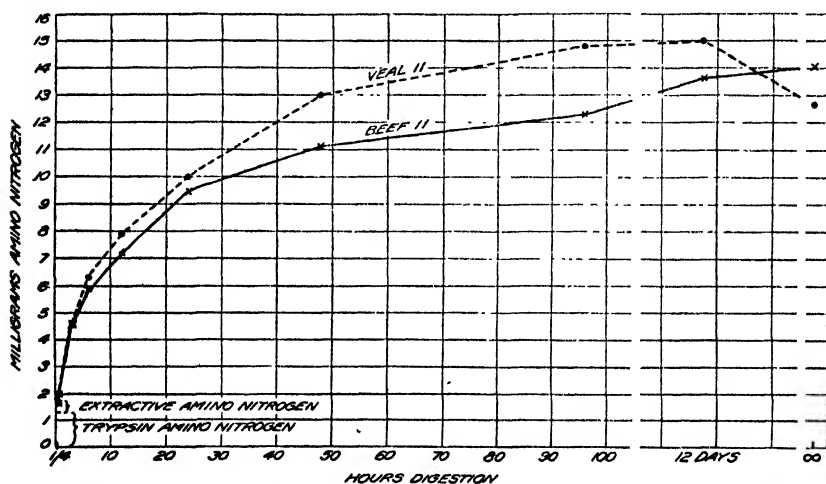


FIG. 4.—Experiment 32. Curve showing the quantity (in milligrams) of amino nitrogen in 10 c.c. of digestion fluid; used 100 gm. of meat plus 2,000 c.c. of 0.5 per cent sodium carbonate plus 4,000 gm. of trypsin 3.

measured was greater in amount than that originally present in the meat. In some of the experiments putrefactive odors were not noticed, although looked for.

The general conclusion drawn from the data of Table XII was the same as that drawn from Tables X and XI—namely, that mature beef and immature veal under the conditions of the experiments were digested by trypsin with equal speed. The slight differences noticed were regarded as physiologically insignificant.

In experiment 27 and its repetition, experiment 28, veal sample 9 was compared with skim-milk sample 2, with the same object as before, to ascertain whether the method would detect a difference in amino-nitrogen liberation where such a difference existed. In both experiments, up to and including the 11-hour determinations, amino nitrogen was liberated in the skim-milk digestion mixtures much more rapidly than in veal sample 9. After this the results were somewhat irregular.

**FREE AMMONIA FORMED DURING DIGESTION.**—Because of the slowness with which ammonia reacts with nitrous acid (see p. 681) it was desirable to determine the amount of ammonia formed during the digestion of mature beef and immature veal and incidentally to ascertain whether the amounts formed were significantly different for the two meats. In experiments 15, 18, 20, 22, and 24, after 24 hours' digestion, 100 c. c. portions of digestion fluid, containing 0.5 gm. of sodium carbonate and corresponding to 5 gm. of meat, were transferred to Kjeldahl flasks, diluted to 500 c. c. with distilled water, and the ammonia distilled into standard acid. The mixtures were quickly brought to a boil and boiled for half an hour. This method is known to give high results, but for the purpose of comparison the errors were negligible. In all cases except veal sample 7 the ammonia obtained neutralized 2 to 3 c. c. of  $N/5$  acid, amounts too small to be a disturbing factor in using the third method or indicating any differences between the beef and veal. From veal sample 7, 7 c. c. of  $N/5$  ammonia was obtained. This animal was sick when purchased (see p. 675). On this score the comparatively high ammonia content of trypsin 2 was a disadvantage.

**BLANKS ON REAGENTS.**—It was found convenient to begin each digestion experiment with fresh alkaline permanganate solution in the absorption pipette and to make blank determinations on the nitrous-acid reagents, water, octyl alcohol, etc., before, during, and after a digestion experiment involving about 20 amino-nitrogen determinations. The blank on the reagents, allowing 20 minutes' reaction time, was 0.6 c. c. nitrogen gas when the permanganate was fresh and rose to 1.2 c. c. after this reagent had been used until absorption had become slow (see p. 680). The smallest volume of nitrogen gas measured in the beginning of a digestion experiment was 3.3 c. c.; the largest, at the end of an experiment, 28.7 c. c.

#### FEEDING EXPERIMENTS ON CATS

In these experiments cats of various ages were fed on a diet in which immature veal was the sole source of nitrogen.

Osborne and Mendel and their coworkers (1914, p. 334) in their investigations emphasize the difference between maintenance and growth. According to these investigators an animal can not maintain its weight unless the diet contains tryptophan, although the diet may be physiologically sufficient in all other respects. Further, an animal can not grow unless lysin is present in the diet, the amount of growth being conditioned by the amount of lysin available. Conversely, the absence of these unique amino acids results in a decline in weight or in stunted growth. According to McCollum and his coworkers (Hart, McCollum, et al., 1911), a diet properly balanced for growth may not be properly balanced for reproduction—i. e., cows fed on either the whole corn plant or the whole wheat plant would grow, but vigorous calves would be produced only by the corn-fed cows.

The principal object of the feeding experiments was to ascertain whether growth and reproduction were possible on a diet in which immature veal was the sole source of nitrogen. The data of the above investigators were used as a guide in planning the experiments.

**DIET.**—The cats' diet consisted of immature veal boiled for one to two hours, to which was added filtered butter fat, sodium chlorid, and calcium carbonate. The immature veal was obtained, as already described, from calves seven days old or less which were killed on the premises. When the meat was trimmed for feeding purposes, the lungs, heart, liver, kidneys, and spleen, together with adherent bits of fat, gristle, etc., were included. For the purposes of the analytic work, digestion experiments, etc., the muscle tissue alone was wanted; for the feeding the intention was to include all parts of the veal that ordinarily are eaten. Thirty-four calves were fed to the cats.

At suitable intervals of from four to seven days about 5 kgm. of veal were removed from the containers in cold storage. After being weighed the meat was cut into pieces about as large as ordinary sugar cubes, transferred to an agate-ware kettle containing about 1 liter of hot water, and boiled for one to two hours. The object was to boil the meat in a small amount of water so that it would be convenient for feeding.

Because of the low fat content of the veal, filtered butter fat was added after the boiled veal had cooled. This was obtained by melting several pounds of butter, allowing the water, casein, etc., to settle to the bottom of the containers, and pouring the supernatant fat through filter papers. The butter fat was kept in bottles in cold storage and used as required. According to Osborne and Mendel (1913, p. 424) butter fat contains no nitrogen. Funk and Macallum (1914) found traces of nitrogen in butter fat, which for the purposes of the present consideration of the diet may be disregarded.

No analyses were made of the materials fed. In a few instances the carefully trimmed muscle tissue used for analyses, etc., was included in the veal diet.

Following were the proportions of the various constituents of the diet:

Immature veal.....	1,300 gm.
Filtered butter fat.....	45 gm.
Calcium carbonate.....	10 gm.
Sodium chlorid.....	10 gm.

The last two constituents were the ordinary "chemically pure analyzed" commercial products. The diet contained no roughage. The above proportions were calculated from the data of Osborne and Mendel (1911, p. 32, 80, 86). Potassium salts and phosphates were omitted, because these were thought to be present in the veal in sufficient amounts.

After the veal had been boiled and the other materials added, the food was kept in an ice box close to the animals' cages. The gelatin present in the food caused the entire mass to become solid, so that there was no loss

through spilling when portions were transferred from the container to the smaller feed pans in the cages. Generally enough food was prepared to last from five to seven days. The ice-box compartment in which the food was kept was also used for the purpose of storing dead guinea pigs, rats, etc., for various biological purposes. Although it was desired to feed the animals with clean food, no unusual precautions were taken. The cover of the can containing the food was seldom tightly in place, and undoubtedly the food was exposed to some extent to bacterial contamination. The conditions under which the meat was kept in cold storage and then boiled were probably better than the conditions in many so-called sanitary kitchens. But the conditions under which the boiled food was stored in the ice box were certainly such as exist in no well-kept kitchen ice box. This was purposely done, in order that the diet actually fed should conform, as nearly as possible, to the poorest rather than the best ice-box conditions for food.

ANIMALS AND ENVIRONMENT.—The animals used in the experiments were ordinary cats, selected at random and brought to the animal room. Some were very young at the beginning of the feeding; others quite old. Their weights are given in Table XIII. After having lived on the immature veal diet for about six months cat 2 was crossed by cat 1, and in due time cat 2 gave birth to a litter of four kittens, given in Table XIII and in figure 6 as cats 5, 6, 7, and 8. One of the kittens (cat 7) died in a few days; the others were nursed by their mother until they could eat the immature veal. It is obvious that since both parents of these kittens had lived and grown on the immature-veal diet for 8 and 10 months, respectively, the birth of these kittens and their subsequent vigorous growth indicated that the diet was entirely satisfactory. There were no indications that toxic bodies were present in the diet or that any of the amino acids essential to normal growth were absent.

TABLE XIII.—Description of cats used in feeding experiments

No.	Description	Weights.			Period of feeding.	Final disposition of animal.
		Initial.	Maximal.	Final.		
1	White male kitten.....	Gm. 695	Gm. 4,080	Gm 3,220	Days. 473	Chloroformed; autopsy performed.
2	Black female kitten.....	837	4,040	2,620	408	Do.
3	Yellow male, old.....	3,605	4,940	4,070	216	Set free.
4	Black male, old.....	3,350	.....	3,960	50	Returned to owner.
5	White male <sup>a</sup> .....	<sup>b</sup> 105	.....	3,080	175	Living in a home.
6	White female <sup>a</sup> .....	<sup>b</sup> 110	.....	2,370	175	Do.
7	Black female <sup>a</sup> .....	<sup>b</sup> 95	.....	100	15	Died; marasmus.
8	Black male <sup>a</sup> .....	<sup>b</sup> 105	.....	2,790	175	Set free.
9	Yellow female kitten.....	580	.....	2,280	114	Do.

<sup>a</sup> Litter produced by cats 1 and 2.<sup>b</sup> At birth.



The animals were kept in cages, singly at first; later, after the kittens had become quite large, they were kept in pairs. The long confinement did not seem to disagree with them. All of the animals were unusually fine in their appearance and disposition, except that toward the close of the experiment cats 1 and 2 apparently suffered from the effects of the long confinement—in their case considerably over a year.

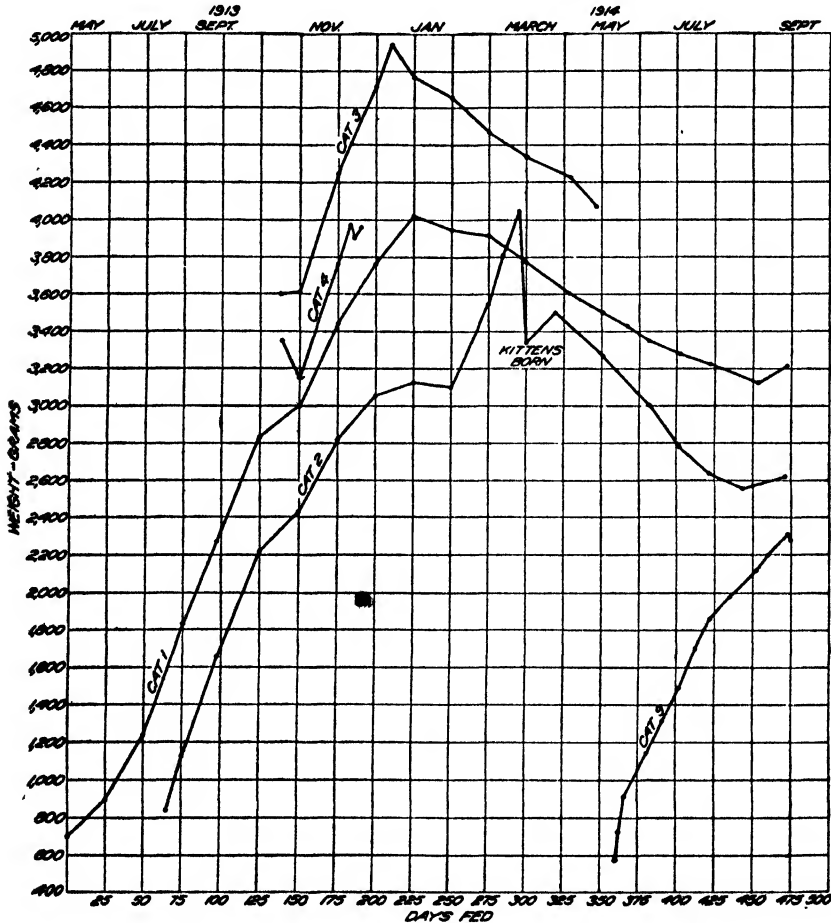


FIG. 5.—Curve showing the rate of growth of cats on an immature-veal diet.

**FEEDING.**—Twice every day, at 9 a. m. and 3 p. m., liberal portions of the veal food were transferred to the feeding pans and placed in the cages. The animals apparently found the food very acceptable in spite of the monotony of the diet. No attempt was made to regulate the amount of food consumed by any animal; they ate as much as they pleased. All of the boiled veal was eaten; not a single lot of the food was found to be distasteful to the animals or in any way noticeably injurious.

**WEIGHTS OF THE ANIMALS.**—The animals were weighed twice every week. The rapid growth of the younger animals and the fattening of the older ones are indicated in figures 5 and 6. The reason for the decline in weight of cats 1, 2, 3, and 4 in the spring and summer of 1914 can not be stated with certainty. The fact that cats 5, 6, 8, and 9, all young, gained weight rapidly on the same diet that the other cats were receiving when they were declining in weight indicated that the loss in weight was not due to the diet but rather to a seasonal variation which affected the weights of the older animals. Cats 1 and 2 were chloroformed at the end of the experiment (September 10, 1914) and autopsies performed by Dr.

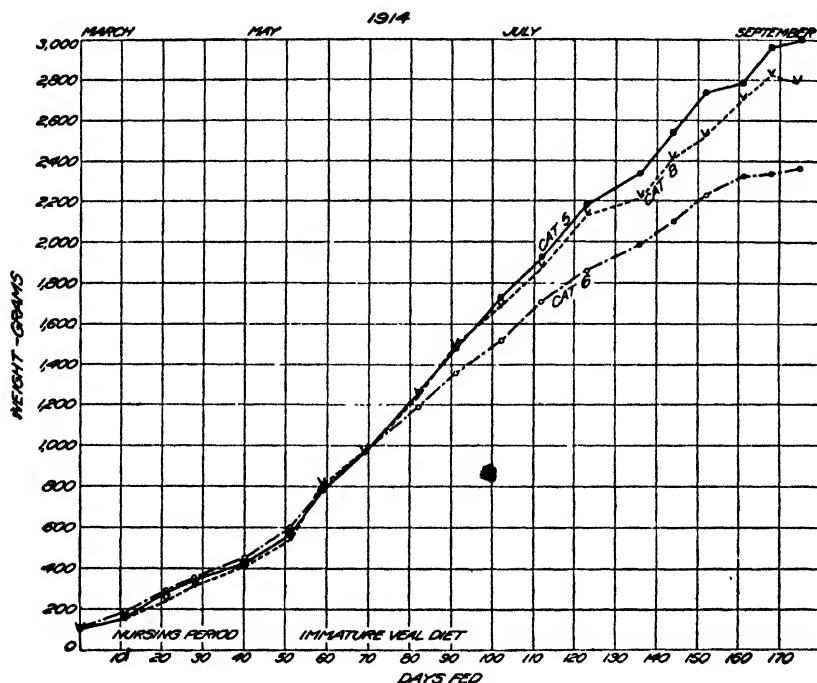


FIG. 6.—Curve showing the rate of growth of newly born cats.

H. J. Washburn, of this division. The animals were found to be in excellent condition, with liberal deposits of fat in both. Apparently the loss in weight in these two animals was due to loss of stored fat. The same was probably true of cat 3, which had the appearance of being unusually fat at the time of its maximum weight.

**CRITERIA OF DIETARY SUFFICIENCY.**—The excreta of the animals were not collected, nor was any chemical work done directly in connection with the feeding experiments. The ability of the animals to utilize the immature veal for the building of their tissues and for the reproduction and nursing of healthy young animals was regarded as a certain indication that the immature veal contained all the amino acids essential to

maintenance, growth, and reproduction. It is true that only one litter of kittens was born, but this would have been practically impossible had an attempt been made to maintain the parents of these kittens for two-thirds of a year on a diet lacking something essential. Cat 2 went through the period of gestation and nursing with every outward indication of excellent health.<sup>1</sup>

#### SUMMARY

(1) During the study of the chemical composition of mature beef and of immature veal, no differences between them that are physiologically significant were detected.

(2) In a large number of artificial-digestion experiments immature veal digested as fast as mature beef. The speed of digestion was measured by three different methods.

(3) Cats were fed on a diet in which immature veal was the sole source of nitrogen. The young animals grew normally on the diet; the older ones became fat. A pair of cats, after living two-thirds of a year on the diet, produced a litter of healthy young kittens which, after the nursing period, continued on the immature-veal diet with excellent growth.

(4) The work indicates that immature veal, when properly prepared, is fit for human food, especially when its deficiencies in fat and possibly in small amounts of undetermined constituents are counterbalanced in the ordinary mixed diet.

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<sup>1</sup> The argument has been offered that the metabolism of the fetus and of the newly born is different from that of older animals and that there is a possibility of toxic substances being present in embryonal or young tissues, which substances, though present in amounts too small to be detected by analytic methods, may be very powerful in their action upon the consumer of very young meat; or, as is sometimes alleged, the newly born animal does not excrete its metabolic end products fast enough, with the result that its tissues are loaded with waste material.

The polypeptid nitrogen which passes unused through the assimilatory system of the fetus or of the newly born is, however, not significant. If by any chance the tissues of a very young calf happened to retain some of its own metabolic products because of retarded excretion or from any other cause whatsoever, so long as the animal was normal otherwise there would be practically no danger to the consumer of such meat from poisonous end products of protein breakdown. However, the tissues of very young calves are not loaded with unexcreted nitrogen. The data obtained on this point are direct and conclusive. (See p. 673.)

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## FACTORS INVOLVED IN THE GROWTH AND THE PYCNIDIUM FORMATION OF *PLENODOMUS FUSCO- MACULANS*.<sup>1</sup>

By GEORGE HERBERT COONS,

*Research Plant Pathologist, Michigan Agricultural Experiment Station*

### INTRODUCTION

The experimentation reported in this paper was begun at the botanical laboratory of the University of Michigan in 1913, continued at the Michigan Agricultural College during the next year, and finally completed in 1915 at the University laboratory.

The fungus *Plenodomus fuscomaculans* was obtained from badly cankered limbs of the apple (*Malus* spp.) which were sent to the Agricultural College laboratory in March, 1911, from Boyne City, Mich. Examination of the cankers at the time of receipt and field studies during the same month showed that the trouble was different from any of the described apple diseases. The cankers showed constant association with a pycnidium-forming fungus. This organism was obtained in pure culture from a single spore, and the causal relation of the fungus to the canker was proved by repeated inoculations and reisolutions. A study of the organism, both on the host and in pure culture, showed that it was a Phoma-like member of the large group Sphaeropsidales, and it corresponded to the species described by Saccardo as *Aposphaeria fuscomaculans*.

The pycnidia, however, show morphological characters by which it is possible to segregate this fungus from the larger, poorly defined genus. These characters, which may be found in the material from the host, become very pronounced in culture. The pycnidia are more or less irregular in shape. The fruiting layer is usually folded so that the chamber is recessed instead of being smooth and regular. The pycnidia are beaked. The wall is composed of two distinct layers and is complete, even at the basal portion. It seems proper to emphasize the morphological character of the wall. Accordingly, the removal of this species from the genus *Aposphaeria* Berk., and the placing of it in the genus *Plenodomus* Preuss,

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<sup>1</sup> Published with the permission of the Director of the Michigan Agricultural Experiment Station.



is proposed. The name of the fungus becomes, under this arrangement, *Plenodomus fuscomaculans* (Sacc.), n. comb.<sup>1</sup>

The present paper deals wholly with the physiological phase of my investigations, the phytopathological studies being reserved for another paper.<sup>2</sup>

The problem consisted of the investigation of the relations of the organism to the environment and the fitting of the environment to the organism—a marked reversal of the common practices in culture making.

#### HISTORICAL REVIEW

The history of the cultivation of micro-organisms is linked with the history of bacteriology and mycology. Progress in these sciences has been largely due to the clarifying effect of pure-culture methods. These originated with the discovery of the method by which media could be sterilized. It is a significant fact, and one which can be traced to the influence of these early experiments, that the solutions and materials used in the first crude cultures were the highly concentrated vegetable and animal decoctions and infusions which experience had shown to be highly liable to putrefaction. Mycology made great advance when, utilizing the newly discovered methods of isolation, the various groups of organisms were brought into pure culture by such masters as Brefeld, De Bary, Hansen, and Zopf. The earlier methods are in vogue to-day in the great bulk of mycological or applied work. In the cultural work of these pioneer studies nutrition was the only factor to which consistent attention was given.

The influence of other factors than nutrition was recognized early, but the methods of culture were varied but little to fit these conditions. Pasteur (1861)<sup>3</sup> showed the difference between aerobiosis and anaerobiosis, but this distinction long remained obscured by the problems of fermentation. The oxygen relations of fungi have been neglected in the ordinary cultural technique, since most fungi tolerate the conditions of the plugged flask or test tube. The sharp temperature requirements of some animal pathogens focused attention upon this factor very early, and accordingly incubators and devices to furnish constant temperature were developed. But there has been wide neglect of this factor. That bacteria grow best in a medium slightly alkaline to litmus and fungi in a medium slightly acid and that this difference can be used to advantage in isolation early became dicta of the sciences. The growth of organisms takes place within such wide limits in composition of culture media and

<sup>1</sup> A discussion of the morphology of this fungus was prepared for the 1915 Report of the Michigan Academy of Science. Delay in publishing this report makes it necessary to give the proposed change in nomenclature in this connection, with only a summary of the reasons for making the change. The latter publication may be looked to for a more complete account of the morphology of the fungus.

<sup>2</sup> The physiological work was suggested by Dr. C. H. Kauffman, of the University of Michigan, and has been done under his direction. I am also indebted to Dr. E. A. Bessey, of the Michigan Agricultural College, for advice and help throughout the investigation.

<sup>3</sup> Bibliographic citations in parentheses refer to "Literature cited," p. 766-769.

under such a range of conditions that accordingly these environmental factors have been neglected in culture work.

The emphasis placed upon nutrition has developed a great body of facts regarding media in which organisms will grow and rules for the preparation of the media. These compositions have the common characteristic that for the most part they present highly concentrated food supplies so complex as to defy analysis. The list includes beef infusion, prune juice, wort, Nähr solution, bread (plain or soaked in sugar solutions), vegetables of all kinds, and the long list of nutrient hydrogels. These media have given excellent vegetative growth; but if the common molds are excluded, it may be said that on the majority of media fructification is the exception rather than the rule.

In recent years many kinds of fruits, vegetables, and other biological products have been tried, either directly or as a base for a nutrient hydrogel. Some of these have produced fructification in forms which had previously grown only vegetatively in culture. Notable examples are corn meal, or corn-meal agar, which in the hands of Shear (Shear and Wood, 1913) and others led to an unraveling of the *Gloeosporium* complex, and oat agar, which in the hands of Clinton (1911) solved the historic *Phytophthora infestans* difficulty.

The complexity of the vast majority of combinations used in contemporary research, however, does not permit the analysis of the contributing factors which lead to fructification. The net contribution, therefore, toward a final analysis, which would furnish a key for unlocking closed approaches with other organisms is small, and further advance, so far as indicated by such work, must be by the same wasteful method of haphazard trial. It is known that organisms will grow under a vast assortment of conditions, but very little is known of the conditions which call out any particular phase of development.

Our knowledge of the physiology of micro-organisms has largely come from a study of their behavior under controlled conditions. The very analytical nature of the type of research used in the study of metabolism has made its methods in sharp contrast with those just described and has made possible evaluation of the various factors involved. The pure-culture methods just discussed and researches on the metabolism of micro-organisms have progressed side by side, and only slightly have the basic principles of the latter been influential in determining the course of the former. The art of cultivating organisms has indeed been developed, but this work is almost wholly empiric; although there is a mass of fundamental facts dealing with metabolism and with the reactions of plants to their environment, these for the most part are totally ignored in ordinary culture methods (Benecke, 1904; Behrens, 1904, p. 436-466).

Studies of the effects of various factors upon the metabolism of fungi naturally were made first with the nutrition of the micro-organisms. It was essential that the work be done with synthetic media; and along

with the development of the various synthetic culture solutions our knowledge of the nutritional requirements of micro-organisms has arisen (Pasteur, 1858, Raulin, 1869, Nägeli, 1880).

The gradual extension of the point of view of physiological response may be considered a guiding principle in cultivating organisms, and after a period of more or less accidental or random application of specific environments to influence growth or reproduction, a definite method based upon this teaching has been developed. Roux and Linossier (1890), with the animal pathogen, *Dematium albicans*, secured marked reactions to specific environmental factors, especially nutrition and oxygen. At the same time Winogradsky (1891) began his well-known work with the nitrifying organisms which he isolated by his method of "elective culture." This method, which consists essentially of so establishing the environment that only organisms of the desired type are able to develop, was carried to great perfection by Beijerinck (1901) with his similar "intensification" method. The bacteria and algæ with which Beijerinck worked required or tolerated different amounts of free oxygen, different nutrition, especially mineral salts, and different temperatures. Beijerinck used these differences as a means of isolation of various forms from a complex substratum (Stockhausen, 1907).

About the same time Klebs began his work on algæ and fungi in pure culture. Where others were concerned with growth, Klebs (1896) made the pure culture answer unsolved questions of life history. He (1913) recognized in the organism definite potentialities—the heredity of the organism. The manifestations of these potentialities are seen in the reactions to environment and in the limits of the various factors tolerated. The particular line of development followed by the organism can be traced to conditions outside of the potentiality, either inner conditions inaugurated by the environmental complex or outer conditions which work through their ability to set up certain internal effects. From this line of reasoning it was but a step to the position that the development of an organism is the resultant of the environment working upon definite internal potentialities of the organism and that with a given potentiality the same external conditions call forth the same response with the constancy of a chemical reaction. This response may be predicted from the type of conditions given, and in this regard Klebs (1900) announced the following propositions, as based upon his work:<sup>1</sup>

1. Growth and reproduction are life processes, which among all organisms depend upon different conditions; among the lower organisms, probably external conditions determine whether growth or reproduction ensue.
2. As long as the characteristic outer conditions for the growth of the lower organisms are present, reproduction does not set in. The favoring conditions for this process are always more or less unfavorable to growth.

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<sup>1</sup> Author's translation.

3. Growth and reproduction differ also in that the working limits of the general life conditions, temperature, oxygen, etc., are narrower for reproduction than for growth. On this account growth can still take place, even if reproduction be limited through too weak or too strong influence of some factor.

4. Growth appears mostly as a preliminary for the initiation of reproduction, and, therefore, as an inner condition for it. Up to a certain limit, not directly growth, but the longer assimilation period is determinative.

From this point of view all the factors which influence life may be considered, and from the basis of the knowledge of their effects on growth, the ultimate effects of these factors upon reproduction may be predicted more or less accurately. This Klebs (1900, 1904) has done in the summary of his contributions to the physiology of reproduction.

Since that time research along this line may be divided into two types of endeavor: (1) Extending the groups to which the laws may be shown to apply and (2) the critical testing of the conclusions with the very organisms with which Klebs worked. The former has extended the limits so that none of the great groups of fungi or algæ are without many examples of the application of the conclusions. The work of the second type has opened up new points of view. Klebs in his experiments used a single strain, and the common experience, in repeating his experiments, is failure until the limits and life relations of the particular strain at hand are known. Accordingly, Kauffman (1908) has emphasized this point in his work with the same species of *Saprolegnia* that Klebs used; but where Klebs worked with one strain, Kauffman used two additional ones; and with this number of forms, each an entity and each varying from the other, Kauffman was able to show that within the limits of each the conclusions were valid. This work emphasizes a point which Klebs has made for his various forms, that each is a specific potentiality, but it makes the specific potentialities innumerable in their scope.

The particular organism with which I worked was one closely related to the large genus *Phoma*. This group, although containing many species, some of great economic importance, had received little attention from a physiological point of view. There have been no attempts to test the validity of Klebs's conclusions for the *Sphaeropsidales*.

Ternetz (1907) isolated from the roots of species of *Vaccinium* and *Oxycoccus* a series of *Phoma* spp. suspected of being mycorrhiza-producing forms. These organisms were grown in pure culture on synthetic media, and their relations to oxygen, nitrogen, and mineral salts were determined with great care. They were found to be sensitive to a restriction of the oxygen supply, especially when growing in a medium poor in nitrogen. These organisms were shown to have the power of utilizing nitrogen from the air. Saida (1902) has claimed the same for *Phoma betae*.

Later, König, Kuhlman, and Thienemann (1911) cultured a species of *Phoma* isolated from water, and although they secured pycnidia in a few

instances, they were unable to determine the conditions under which fruiting bodies developed, but they surmised that probably the lack of food supply was the causal relation.

Other related genera have been studied more or less, and detailed accounts of the growth and fruit-body formation of several species of *Phomopsis* on the ordinary laboratory media have been given. (Roberts, 1913; Harter and Field, 1913; Harter, 1914.)

*Plenodomus destruens* has recently been described by Harter (1913), who has cultured the organism upon the ordinary laboratory media, and has determined its optimum temperature. For the most part the above-mentioned articles, written from a phytopathological point of view, have used the pure culture as a device for furnishing material for pathogenic studies, and the description of the organism in culture is largely for diagnostic purposes.

#### METHODS OF INVESTIGATION

As *Plenodomus fuscomaculans* had shown no form of reproduction under the ordinary methods of culture (see p. 724), it seemed to afford an excellent opportunity to try the effect of various environmental factors as a test of the applicability of the methods of Klebs to phytopathological studies.

The strain of the organism used was the progeny of a single pycnidiospore, isolated by the dilution method. This strain had been tested and was known to be pathogenic to apple. In 1913 another isolation was made from a second collection of material, and a second strain obtained and similarly tested. In all later work both strains were used in all experiments. Aside from slight differences in vigor of growth, the cultures gave the same reactions.

All experiments were made in duplicate with each strain; hence, the experiments reported give results which are a summary from the record of at least two, and, in most cases, of four parallel cultures.

The glassware used, unless otherwise indicated, was the ordinary German glass. All glass culture dishes, when other than tap water was to be used, were cleaned by immersion overnight in cleaning fluid, followed by four rinsings of tap water and one rinsing of distilled water. When water of a higher purity than ordinary distilled water was to be used in the medium, the vessels were given an additional rinsing with the purer water.

The most commonly used culture dishes were small glass preparation dishes, or capsules, of about 35 c. c. capacity. These had a loosely fitting cover which rested upon a shoulder of the bottom.

The chemicals used were those of Kahlbaum. Solutions of various chemicals were made up as weight-normal solutions (1 molecular weight in grams in 1 liter of water); and where chemicals contained water of crystallization, this was added in computing the molecular weight.

The various nutrient media mentioned were made according to the ordinary formulæ. Prune-juice agar was made by using 75 gm. of prunes with 20 gm. of agar per liter. Pea, corn, and oat broth were made by autoclaving two seeds or grains of each in 10 c. c. of distilled water.

The tap water used in some experiments had a conductivity of approximately  $400$  to  $600 \times 10^{-6}$ , while the conductivity water averaged  $2 \times 10^{-6}$  at the time of preparation. This water was obtained either by distilling ordinary distilled water in a block-tin still or by double distilling such water in Jena glass. As is generally recognized, ordinary distilled water varies greatly in quality, but the conductivity of the distilled water used was probably within  $4$  to  $12 \times 10^{-6}$ .

The filter paper used was Schleicher and Schull's, and, unless otherwise given, was No. 595. All media were autoclaved at approximately 15 pounds for 10 to 15 minutes, unless otherwise stated.

Inoculations, unless specified otherwise, were made with one drop of a spore suspension obtained by crushing pycnidia in a water blank. This was then filtered through a filter paper into a sterile test tube. The filter paper was sterilized in a test tube drawn out to make a funnel. This gave a device by which large masses of mycelium and pycnidia walls could be strained from the suspension. The spore suspension was added to the various cultures by means of a sterile bulb pipette equipped with a long, small-bore outlet.

#### EARLY EXPERIMENTS WITH ORDINARY LABORATORY METHODS

The organism brought into pure culture was grown upon ordinary laboratory media. This work was done in the spring and fall of 1911 at the Michigan Agricultural College, at a table at the rear of a large laboratory lighted from one side. Cultures were made in Petri dishes, flasks, and test tubes. Standard agar, prune-juice agar, apple stem and bark agar, apple twigs, parsnips, corn meal, potato, carrot, bean pods, beef broth, and filter paper, without other nutrients, as well as with various nutrient solutions, were the media employed. Cultures were grown under a variety of conditions, such as room conditions (test tubes in cans or in wire baskets), in the incubator at  $25^{\circ}$  C., and in the ice box at temperatures ranging from  $7^{\circ}$  to  $13^{\circ}$ . A few cultures were grown at  $37.5^{\circ}$ . On all the media mentioned growth was obtained, with more or less difference in color or vigor, but in no case were fruiting bodies of any sort produced. In some cases the cultures were allowed to dry out gradually; in other cases sterile water was added from time to time. Flasks of corn meal, with an abundant water supply, were set away in a cupboard for three months in an attempt to secure fruiting bodies in the time-honored way. In spite of this variety of trials, the organism remained a typical "sterile fungus," of which a number have been reported in literature.

But the organism, when inoculated into the host, gave characteristic lesions and typical pycnidia from which the organism could again be isolated. These reisolutions were repeatedly tested, with results parallel to those obtained from the parent culture. Certain fungi—e. g., *Botryosphaeria ribis* and *Rhizoctonia* spp.—are known to fruit exclusively upon the host, and evidence seemed to point to this organism as one of that type.

#### EXPERIMENTS UNDER CONTROLLED CONDITIONS

In 1913, experiments were begun at the University of Michigan laboratory. In this work an attempt was made to find the effects of varying environmental factors, or, in other words, to analyze the formative as well as the inhibiting factors involved in growth and reproduction.

#### CONDITIONS FOR GROWTH AND REPRODUCTION

##### PHYSICAL FACTORS

##### LIGHT

The influence of light upon organisms has been recognized for a long time. Fries (1821) and the early authors attributed great morphogenic power to light. They found their greatest substantiation of the effect of light upon organisms in the excessive growth of mycelium in caves, accompanied, as it was, by the suppression of fructification. The literature is full of these observations, many of which are quoted by Elfving (1890). Scientific experiment with light as a factor influencing growth and reproduction of fungi began with the classic studies of Brefeld (1877, 1881, 1889) on *Coprinus* spp. Brefeld found in some species a complete suppression of fructification when cultures developed in the dark; in other species fructification took place, but the growth was puny. In some the high temperature of the summer replaced in part the beneficial effect of light. In a set of interesting experiments Brefeld showed that the exposure of mycelium to light need not be long (two to three hours) in order to have fructification begin, and that cultures so exposed developed normally, although in the dark. The work of Brefeld substantiated that of the older observers. Lakon (1907) has attempted to show that the action attributed to light is really due to transpiration differences in the cultures of *Coprinus* spp.

Downes and Blunt (1878) had previously experimented with the effect of light upon bacteria and found that it had a very detrimental effect upon these organisms. This they attributed to the action of the ultraviolet rays in augmenting oxidation, a property of light long recognized by chemists. Their conclusion was later substantiated by Ward (1893).

Elfving (1890) gave the results of his experiments with light in a monograph on the subject. Searching the literature, the only important experimental work found was that of Brefeld (1877, 1881, 1889) already

mentioned. Many had studied the effect of light upon germination, but the varying intensities of light used, etc., yielded nothing in the way of a generalization.

Elfving (1890) sought to find the influence of light upon metabolism. He used cultures of *Penicillium* spp. and a related fungus (*Briaraea* sp.) growing in a synthetic solution. He used several sources of carbon and nitrogen. Basing his conclusion upon the dry weights obtained in the light and in the dark, he decided that light acts upon fungi as an inhibitor of organic synthesis. The closer the food material is to protoplasm in its make-up, the less the light inhibits. This produces the result which he finds analogous to conditions in the higher plants—that light restricts vegetative growth. Elfving, in view of the great similarity of fungi in their physiological relations, boldly makes his conclusions apply to the whole group of fungi.

Lendner (1896) tested the effect of light upon species of *Mucor*, *Botrytis*, *Amblyosporium*, and *Sterigmatocystis*, finding that light was effective only under conditions of unfavorable nutrition.

Finally, in the experiments of Ternetz (1900) with *Ascophanus carneus*, asci were produced only under the influence of light.

Light is seen to be a factor of widely varying importance for organisms, although the effect on vegetative growth is commonly shown to be prejudicial. For some it is a morphogenic factor of great influence; for others it is of no moment.

Pure cultures of the organism on prune-juice agar and on parsnip had been brought from the Agricultural College laboratory. At Ann Arbor these cultures began to produce pycnidia in a few days. When analyzed, this striking behavior showed that light was probably the factor concerned with the fruit-body formation. The following experiments were started to test the validity of this inference. While work at the Agricultural College had been done some distance from the window (25 to 30 feet), the cultures at Ann Arbor were placed a few feet from a south window in strong diffuse daylight, and at times in direct sunlight.

Experience had shown that the organism would make a fair growth on filter paper. Filter-paper disks, about 5 cm. across, were folded to form cones, and these were set up in 10 c. c. of tap water in preparation dishes. These were autoclaved. To some, one drop (1/20 c. c.) of a sterile *M/I* chemical was added, as indicated in Table III. The preparation dishes were inoculated with a mycelium suspension, and were placed in tall battery jars covered with filter paper. One set of cultures was placed in a light-tight cupboard, while the other was left upon the table in strong diffuse light. Thermometer readings showed at times of strongest light that the illuminated cultures were 2 degrees centigrade warmer than those in the dark. Readings were made in nine days.



TABLE I.—*Effect of light: Tests with filter paper (readings in 9 days)*<sup>1</sup>

Conditions.	Number of pycnidia.	Growth.
Filter paper in light.....	+	++
Filter paper in dark.....	—	++ <sup>2</sup>

<sup>1</sup> In tables where a single plus symbol (+) is contrasted with the negative sign (—), presence or absence is meant. Where a series of readings is given and several plus symbols are used with reference to pycnidia production, they give the average of two and at times of four readings, as follows: + = 1 to 10 pycnidia; ++ = 10 to 25; +++ = 25 to 50; ++++ = 100. As applied to growth the same plus symbols mean, respectively, scant, fair, good, abundant growth.

<sup>2</sup> A trifle stronger than above.

The cultures which had been in the dark were exposed to light about an hour at a time, when the reading was made. A second observation after 27 days showed the following result:

TABLE II.—*Effect of light: Tests with filter paper (readings in 27 days)*

Conditions.	Number of pycnidia.	Growth.
Filter paper in light.....	+	++
Filter paper in dark (except one hour's exposure). . .	Sclerotia.	+++

These bodies, called provisionally "sclerotia," when examined under the microscope were found to be minute brown bodies about one-tenth the size of the ordinary pycnidium and consisted of a firm, solid pseudo-parenchyma.

In no case was any suggestion of chamber formation noticed; nor were any spores found. It is noteworthy that the growth after this longer period could be seen to be stronger in the dark than in the light.

As part of the same experiment, a drop of some sterile *M/1* chemical was added as indicated to a number of similar filter-paper cones. The results are as follows:

TABLE III.—*Effect of light: Pycnidium formation on filter paper plus various chemicals*

Chemical.	9 days.		27 days.		40 days.	
	Light.	Dark.	Light.	Dark.	Light.	Dark.
Filter paper + approximately 1/20 c. c. of—						
Calcium nitrate, Ca(NO <sub>3</sub> ) <sub>2</sub> <i>M/1</i> .....	+	—	+	Sclerotia.	+	Sclerotia.
Potassium acid phosphate, KH <sub>2</sub> PO <sub>4</sub> <i>M/1</i> ..	+	—	+	—	+	+
Potassium nitrate, KNO <sub>3</sub> <i>M/1</i> .....	+	—	+	—	+	Sclerotia.
Calcium acid phosphate, Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> <i>M/1</i> .....	+	—	+	—	+	—

At the time of making the first reading, the cultures were exposed to the light for about an hour, and at the second reading they were exposed to strong diffused daylight for two hours.

From a consideration of the experiments reported in these tables, it is evident that light is a factor directly concerned with pycnidium production. There is also a strong tendency toward increased growth in the dark.

The experiment has been repeated many times, with a great number of duplicate cultures (60 in one instance), and always with similar results. The following is a typical experiment. Preparation dishes with water distilled out of sulphuric acid and filter paper and with water alone were inoculated with spores of each of the two strains of the organism. One set was wrapped in a double thickness of paper such as is used in photographic film rolls. The dishes exposed to light were set in glass battery jars on the window sill. The light was made diffuse by a sheet of yellow manila paper tacked on the window. The dark cultures were set away from the window in the interior of the room. The difference in temperature was the reverse of the conditions in the preceding experiments, since closeness to the cold window more than compensated for the effect of the light. In this experiment after a month no pycnidia formed in the dark, while in every culture in the light numerous pycnidia were found.

TABLE IV.—*Effect of light: Test with two strains of the organism*

Strain and conditions.	Pycnidia.		Growth.	
	Light.	Dark.	Light.	Dark.
Strain I:				
Filter paper + water.....	25	o	++	++++
Double-distilled water.....	2	o	+	+
Strain II:				
Filter paper + water.....	11	o	+	+
Double-distilled water.....	o	o	+	+

To avoid the criticism that the results observed were due to differences in aeration brought about by wrapping the capsules, or by the use of the dark closet, and to test other conditions of food supply, cultures were made with corn broth, and these were placed in a specially constructed light-tight box, which, however, allowed aeration. The box was made of two tubes of different diameters (7 and 9 inches), one inside the other. These cylinders were each 12 inches tall and toothed at the ends. A pair of caps were made for these cylinders. The caps consisted of a disk of paper about 10 inches in diameter, and a short cylinder 8 inches in diameter was glued to it. The joint was made light-tight with black paraffin. When these tall cylinders were set up with the cylinders of the caps fitting between them, light was excluded. The cultures were

placed in battery jars. The toothed tops of the cylinders allowed a circulation of air. For tests with light, the cultures were ordinarily placed in a battery jar and covered with filter paper or cloth to protect them from dust. As a further safeguard from error, however, a similar container was made, but with celluloid substituted for black paper.

The result with corn broth, after three weeks, is given in Table V.

TABLE V.—*Effect of light: Test with corn broth in light-tight box*

Conditions.	Pycnidia.	Growth.
Light in battery jar.....	+++	Fair.
Light in celluloid chamber.....	++	Fair.
Dark in black-paper chamber.....	—	Strong.

From this experimentation it is evident that light is a determining factor for pycnidium formation in this organism, irrespective of the type of nourishment, and that the action of light is distinct from effects which might be attributed to faulty aeration in the darkened cultures. The slight depression of pycnidia formation in the slightly darkened celluloid chamber is significant. Growth is increased in the dark.

Cultures on corn broth, in both light and dark, were subjected to a variety of air conditions. Stopped flasks were fitted with two glass tubes, one of which extended to the surface of the culture, the other merely through the cork. As indicated in Table VI, some were connected with the water pump and filtered air which had bubbled through water was gently drawn through. As a check, some flasks were left with no additional circulation, while some were plugged with cotton.

TABLE VI.—*Effect of air circulation: Test with corn broth in stoppered flasks*

[Time, 1 month<sup>1</sup>]

Conditions.	Pycnidia.	Growth.
Attached to aspirator:		
Light.....	++++	++
Dark.....	—	++++
Air only through small tubes:		
Light.....	++	++
Dark.....	—	+++
Flasks plugged with cotton:		
Light.....	++++	++
Dark.....	—	+++

<sup>1</sup> The experiment was continued a second month with no change in relative values.

This experiment eliminates any possibility that the effect attributed to light may have come from faulty aeration or deficient transpiration. The experiment further has significance from the point of view of aeration.

The production of sclerotia, as recorded in Tables II and III, after a short exposure to light, and the production of pycnidia in one case, where the exposure was not more than two hours, suggested that the exposure to light did not need to be of long duration in order to produce its morphogenic effects. The capsules of a preceding experiment, which had shown no pycnidia after three weeks in the dark chamber, were divided into series, one of which was exposed to strong diffuse light on the window sill for two hours, while the other series was continued in the dark box. The exposed cultures were returned to the box, and after a week the cultures were examined.

TABLE VII.—*Effect of light. Continued test with corn broth*

Corn broth.	Mature pycnidia	Growth.
Dark.....	0	Aerial growth.
Dark, light (2 hours), dark. . . . .	3-4	Aerial growth checked, mycelium matted.

Pycnidium production had not increased upon a second examination a week later.

This experiment teaches that pycnidium formation is not only associated with light, but that the effect of light is to inaugurate a type of growth which can proceed to completion even in the absence of light. But after exposure to light the number of fruiting bodies formed is limited and the process does not continue to the production of a large number of fruiting bodies.

To summarize the results of this series of experiments, it may be pointed out that light is a decisive factor, which determines, in certain cultures, whether reproduction takes place or not, and that the action of this factor is irrespective of the richness or the poverty of the substratum in nutrients. As a morphogenic factor, its action is to inaugurate fruit-body formation, but it is not essential to the process, once inaugurated. Associated with its effect in initiating reproduction, we have its repressing effect on growth.

All subsequent cultures made with the organism had good exposure to strong diffuse light, unless otherwise expressly stated.

#### TEMPERATURE

It has been said that the influence of temperature was very early recognized in its influence on the life processes of fungi. Raulin (1869) in his studies of *Aspergillus niger* grew the organism at the most favorable temperature—33°. Wiesner (1873) very early formulated the behavior of *Penicillium glaucum* by a law which took into account that the time necessary for fructification did not depend wholly upon the

temperature at which a culture was placed, but depended also upon the temperature at which the organism had developed, which is, of course, a way of saying that the process of fruit-body formation is a process which depends upon the previous metabolism, and that conditions which delay the latter react similarly upon the former. The literature teems with individual facts about the temperature relation (Behrens, 1905, p. 444-449). The temperature relation, better than any other, shows the significance of the cardinal points in relation to life processes. Accordingly, we have the generalization of Klebs (1900), that the limits permitting vegetative growth are wider than those permitting fructification, and this law is nowhere more admirably illustrated than in the temperature relation.

My early experiments with temperature are not applicable, because light was excluded. Experience had shown that pycnidia were formed at the ordinary limits of room temperature. Successful cultures on various sorts of media were made in the winter with the average room temperature, 20 to 23°, and in the summer with a temperature range from 25 to 30°, so long as the light factor was not neglected.

A series of temperature experiments was made with the synthetic solution described upon page 752 in 100 c. c. flasks. These flasks were inoculated, and after three weeks' growth in weak diffuse light were subjected to the temperature indicated.

TABLE VIII.—*Effect of temperature*

Temperature.	How obtained.	Number of pycnidia.	Increase in growth.
°C.			
6-6½. ....	Constant temperature ice box with glass doors . . . . .	o	Slight.
10-12. ....	Located at window in cold hallway . . . . .	+	Fair. <sup>1</sup>
20-22. ....	Room temperature near window . . . . .	+	Strong.
23. ....	Constant temperature incubator, outer door open, glass door closed	o	Weak.
33. ....	do . . . . .	o	Do.

<sup>1</sup> Pycnidia began to form after a week.

The varying conditions in this experiment make necessary some interpretation for the clearing away of the apparent contradictions in the results. The absence of pycnidia in the 23° and 33° incubators, which is in seeming contradiction to the production of pycnidia in the summer time, or even at ordinary room temperature, was doubtless due to the fact that either the light was too much reduced or the air was depleted of oxygen. That the former influence was not operative seems likely from the fact that cultures standing in battery jars upon the incubator had at another time produced pycnidia. The incubators contained other cultures at the time of the experiment, and, although the doors were opened from time to time, the chamber had the ordinary strong odor of old cultures. The constant low-temperature chamber

which was designed especially for this work seems free from this criticism, since cultures placed in it before icing began developed pycnidia. This incubator had two openings (1-inch diameter) to the outside and a small fan, driven by a motor, which continuously brought about good aeration and prevented fogging of the doors. The constancy of temperature during the first week can be vouched for within the limits set, and for the next month no large deviation occurred.

The lack of apparatus to give constant temperatures, and at the same time illumination and aeration, prevented any further experimentation along this line. Pycnidia have been obtained in cultures with a temperature range of from 10° to 30° C. No pycnidia were obtained at 6° C. and no other inhibiting factor than temperature is known to have entered. The experiments with the constant-temperature incubators are disregarded because of the entrance of other factors, but are included merely to show the difficulty of experimenting with this factor.

The wide limits of pycnidium production, so far as temperature is concerned, allowed great leeway in experimentation; but outside these limits temperature may show as marked an effect as light. It is noteworthy that growth shows wider temperature limits than reproduction.

#### AERATION

The oxygen relation is no doubt the most essential of all life relations, and the statement "No life without air" has been shown to be universal, the contributions of Beijerinck (1893), as well as those of Fermi and Bassu (1904, 1905), showing that even the strictest of known anaerobes require minute traces of free oxygen. The relation of oxygen to plants was recognized almost from the beginning, but the interpretation of respiration by Pfeffer (1889) is fundamental. In this we have respiration portrayed as the energy-releasing process. Subsequent work has dealt with the effect of various external conditions upon the respiratory quotient. Necessarily all respiration relations depend upon the quality of the nutrition as well as the quantity of nutrients. The general conclusion which has been expressed by Beijerinck (1899), that all plants have a definite oxygen optimum and that aerobes are those whose optimum is high, while anaerobes are organisms whose air requirement is low, seems to summarize most nearly the numerous contributions.

The limiting effect of scanty aeration upon reproduction has already been mentioned. Determination of the potency of this factor in any but general ways is difficult, because of other factors involved.

Observation very early showed that greater pycnidium production took place in a capsule or Petri dish than in a plugged test tube, and that small test tubes were not so effective for pycnidium production as larger ones. Similarly, when capsules were piled one on top of another in a battery jar, pycnidia production took place in the top capsules first, although in a few days or a week pycnidia were formed in all.

If a vigorous culture on suitable media (prune-juice agar or corn-meal agar) was sealed with sealing wax no pycnidia were produced, even though comparison tubes unsealed produced pycnidia in abundance. Sealed tubes which had remained without pycnidia for two weeks had the sealing wax removed, and the pycnidium formation was slowly inaugurated. Corn broth in capsules, if covered with a small bell or if placed in a battery jar with a tight-fitting ground-glass cover, produced scanty mycelial growth but no pycnidia.

Tests for aerotropism were made with spores in melted agar. Melted agar was heavily sown with spores of the organism. Some tubes were prepared with a lighter seeding. Small drops of these agars were placed on sterile slides and sterile cover glasses pressed down upon them. Other preparations were made with the cover glass tilted, as in Beijerinck's (1893) well-known experiments. These slides were put away in a moist chamber for 24 hours at ordinary room temperatures. The results of these tests were extremely interesting.

Where the spores were numerous those at the center of the preparation showed no evidences of germination other than a slight swelling. Outside the center zone germination became more and more evident. About 5 mm. from the edge of the cover glass the germ tubes were found to be 10 to 50 times the length of the spore. At the edge of the cover glass the germ tubes had extended outward nearly a half of a millimeter. Where the spores were fewer in number the germination in the center sometimes proceeded to the extension of a short germ tube. There was no evidence of a definite tropism toward the border of the cover glass, but frequently the same spore would have sent out two germ tubes from opposite sides, one growing toward the edge of the glass, the other growing inward. Then it was noticed that the sprout growing in the medium with the richer oxygen supply was from 4 to 10 times the length of the other germ tube.

Where a clump of spores occurred about halfway from the center to the edge of the cover glass, those spores near the edge swelled strongly and put out germ tubes, while spores of the same clump, situated nearer the center, remained dormant, or at least swelled only slightly. The repression of germination in these spores seemed to be related to the scanty oxygen supply, and for this there was strong competition.

A series of flasks of different sizes was prepared with filter-paper cones, wet with 5 c. c. of distilled water. These were autoclaved and inoculated with a spore suspension. Immediately after inoculation the cotton plug was pushed slightly down the neck of the flask and the flasks were sealed with melted paraffin. The flasks were set in a window in even, diffuse illumination. After a month the reading shown in Table IX was obtained.

TABLE IX.—Effect of aeration: Test with flasks of different sizes

Size of flask.	Number of pycnidia.	Growth.
<i>C. c.</i>		
50.....	.....	None.
100.....	.....	None.
250.....	.....	Doubtful.
500.....	o	Weak.
1,000.....	o	Fair, mycelium blackish.

There were no checks in this experiment, but the behavior of this organism on filter paper had been so constant as to leave little doubt of repression of pycnidia having taken place, owing to the sealing of the flasks.

A similar experiment was performed with a number of nutrient solutions, some of which were known to allow pycnidium production, and others of which were known to yield only strong growth. Ten c. c. of each solution were used. This experiment was done in duplicate and was carefully checked. Inoculation was made with small masses of mycelium. The flasks, after inoculation, were sealed and stood in strong diffuse light upon a table. Table X gives the summary of this experiment.

TABLE X.—Effect of aeration: Tests with various nutrient solutions

[Time, 1 month]

Solution.	Size of flask.	Sealed.		Check.	
		Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
Raulin solution <sup>1</sup> (levulose substituted for sucrose).	C. c.				
	900	o	Heavy mat. . . . .	o	Heavy mat.
	500	o	Heavy mat . . . . .	o	Heavy mat.
	125	o	Fair. . . . .	o	Heavy mat.
	50	o	Scant. . . . .	o	Heavy mat.
Acid Dox <sup>2</sup> solution with 1 c. c. of glycerin added to each flask.	900	o	Scant. . . . .	o	Fair, white.
	500	o	Scant. . . . .	o	Fair, white.
	125	o	Scant. . . . .	o	Fair, white.
	50	o	None. . . . .	o	Fair, white.
Alkaline Dox solution with 1 c. c. of glycerin added to each flask.	900	o	Very scant. . . . .	o	Weak.
	500	o	Very scant. . . . .	o	Weak.
	125	o	Very scant. . . . .	o	Weak.
	50	o	None. . . . .	o	Weak.
Raulin solution. . .	900	o	Fair. . . . .	o	Heavy mat.
	500	o	Fair. . . . .	o	Heavy mat.
	125	o	Fair. . . . .	o	Heavy mat.
	50	o	Fair. . . . .	o	Heavy mat.

<sup>1</sup> Raulin solution: 1,500 parts of water; 70 parts of cane sugar (35 gm. levulose); 4 parts of tartaric acid; 4 parts of ammonium nitrate; 0.6 part of ammonium phosphate; 0.4 part of magnesium carbonate; 0.6 part of potassium carbonate; 0.25 part of ammonium sulphate; 0.07 part of zinc sulphate; 0.07 part of iron sulphate; 0.07 part of potassium silicate.

<sup>2</sup> Dox solution, etc. (Czapek): Distilled water (H<sub>2</sub>O), 3,000 c. c.; magnesium sulphate (MgSO<sub>4</sub>), 1.5 gm.; dibasic potassium phosphate (K<sub>2</sub>HPO<sub>4</sub>), 3.0 gm.; sodium chlorid (KCl), 1.5 gm.; ferrous sulphate (FeSO<sub>4</sub>), 0.03 gm.; with Potassium acid phosphate (KH<sub>2</sub>PO<sub>4</sub>), acid solution (Thom, 1910).



TABLE X.—Effect of aeration: Tests with various nutrient solutions—Continued

Solution.	Size of flask.	Sealed.		Check.	
		Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
Raulin solution + $\frac{1}{2}$ c. c. M/1 calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ ).	C. c.				
	900	0	Fair, mat....	10	Fair, thin mat.
	500	0	Fair, no mat....	10	Fair, thin mat.
	125	0	Fair.....	25+	Mat.
	50	0	Fair.....	25+	Mat.
Raulin solution with levulose, on filter paper cones.	900	0	Filter covered....	0	Paper covered.
	500	0	Filter covered....	0	Paper covered.
	125	0	Less than above....	0	Paper covered.
	50	0	As above....	0	Paper covered.
Acid Dox solution + 1 c. c. M/10 arabinose.	900	5	Scanty white.	25+	Fair, white.
	500	1-2	Scanty white...	25+	Fair, white.
	125	0	Scanty white....	50+	Fair, mat.
	50	0	Scanty white.	50+	Fair, mat.
Acid Dox solution + 1 gm. potato starch.	900	?	Strong.....	20+	Strong, mat.
	500	?	Strong.....	20+	Strong, mat.
	125	0	Fair.....	20+	Strong, mat.
	50	0	Fair.....	20+	Strong, mat.

This experiment shows the effect of scanty aeration in repression of growth as well as an almost complete suppression of pycnidia in the sealed flasks. In the two cases where pycnidium production did take place in the sealed flasks, the fructification occurred in the larger flasks of the series. It must be said that the check flasks, especially the larger sized ones, were almost dry at the close of the experiment and the humidity conditions as well as the concentration were different from those of the sealed flasks. For the first three weeks, however, the cultures were approximately the same, and it seems safe to attribute the difference in growth and pycnidium suppression to improper aeration, rather than to the drying or concentration, especially since, as will be seen from later experiments, these factors play but little part in pycnidium production.

From the many observations recorded here, and from the experiments, it seems safe to conclude that this organism is very sensitive to the oxygen supply, and it requires good aeration for optimum growth and for pycnidium production.

#### HUMIDITY (TRANSPIRATION)

From a number of indications in cultures, it was felt that transpiration might be a factor of more or less importance in the growth and reproduction of this fungus. A study of the literature dealing with reproduction, especially the work of Klebs (1898) with *Sporodinia grandis*, made this

seem extremely probable. It was seen that cultures on various complex media did not produce pycnidia until they began to dry out, as a general rule. Moreover, on nutrient solutions the pycnidia commonly form on the surface. On vegetables, such as carrot or parsnip, or on prune-juice agar, the pycnidia formed in the aerial mycelium.

Very early this relation was suspected as being operative, and the filter paper cone was used in the first experiments to further transpiration and aeration. When, however, the relation was tested, it was seen that the actual formative influence of transpiration had been greatly overestimated. Filter-paper cones were compared with similar-sized disks of filter paper entirely submerged. Inoculation was made with bits of mycelium, and the cultures stood on the table in strong diffuse light.

TABLE XI.—*Effect of humidity: Test with filter paper*

[Time, 1 month]

Conditions.	Number of pycnidia.	Growth.
Cones mostly above water. ....	5-10	Fair.
Submerged paper. ....	10+	Scanty.

It is seen that the pycnidia production goes on after this period as strongly, if not better, in the submerged condition, while the growth seems slightly stronger on the cone. Since differences of this sort are hard to estimate, little importance is attached to the slight differences. Nevertheless, we have in this experiment striking evidence that under conditions where transpiration is reduced to the zero point pycnidium production is nevertheless vigorous.

In this experiment the possible relation to contact stimuli is not avoided. The following observation is even more conclusive, for here contact relations are limited to the effect of mutual contact of the threads of the mycelium itself, and no further elimination of a hypothetical contact relation is possible. Several water blanks of ordinary distilled water were heavily inoculated with spores and mycelium, respectively. After a month the following observation was recorded:

TABLE XII.—*Effect of humidity: Test with inoculated water*

Form of inoculation.	Number of pycnidia.	Growth.
Spores. ....	4-10	Fair amount of white, byssoid mycelium. Total submergence.
Mycelium. ....	2-10	Fair amount of white, cottony mycelium. Total submergence.

From these experiments there can be little doubt that pycnidia can be produced by this fungus without reference to the factor of transpiration.

We now come to an experiment in which the time element was recorded and in which the influence of a number of different degrees of air humidity was tested.

Four bell jars with a hole in the top were connected with a compressed-air reservoir so that a gentle current of air could be sent through the apparatus. The air was led into the bell jars by a tube reaching to the bottom of the bell jar and taken out by short tubes which extended through the stopper but a short distance. To secure moist conditions the air was bubbled through distilled water, while dry air was obtained by sending the blast through two towers filled with calcium chlorid. The first bell jar received moist air constantly, the fourth dry air constantly, and the second and third were connected by Y tubes to both the dry and wet bell jars, so that they could be made to receive either wet or dry air independently. Throughout the experiment the conditions in these two bell jars were alternated. The second bell jar received wet air for three days and then dry air for one day, while the conditions were reversed for the third jar. Preliminary tests with a Lamprecht polymeter in each jar (these were set to agree with a sling psychrometer reading) showed that the humidity within the first jar ranged from 65 per cent to 70 per cent, and in the fourth the humidity was only 20 per cent. In the other bell jars a humidity of 65 per cent or a dryness corresponding to 25 per cent could be obtained in a half hour by blowing in wet or dry air. The blast was almost continuous throughout the experiment except for a period each day between about 3 a. m. and 8 a. m., at which time the pressure was lacking. The bell jars giving wet conditions were fogged at times, but, as the apparatus was in strong light and as the fog disappeared except when the bell jars were hit by a cold draft, it is very likely that the light intensities were sufficient in all cases. For media various substances were used. Bits of pear and apple twigs, corn meal, slices of carrot and apple, peas, rice, and corn, as well as corn-meal agar and glucose agar, were autoclaved. The media were prepared in capsules without the covers and were placed in tiers in round wire baskets so that each capsule had free access to air. The basket was slipped inside a battery jar and was covered with a cotton pad held in place by a glass plate. Five sets of this sort were prepared, four to be subsequently placed under the bell jars, and the fifth to be used as a check without aeration. The media were autoclaved and then inoculated with a drop of spore suspension to each dish. The cultures were left one week under ordinary room conditions. At the beginning of the test of the various air conditions the bell jars were drenched with solution of mercury bichlorid. The basket was lifted under aseptic precautions and set upon a small metal rack. This

rack, which had been previously disinfected, rested upon the ground-glass base. The bell jar was quickly put in place over the basket and sealed air-tight by the use of anhydrous lanolin. Since the air pressure at times amounted to several pounds, these bell jars had to be clamped to the base plate. This was accomplished by boards drilled at the corners, the top one fitted with a 3-inch hole, through which the top of the bell jar projected. Long bolts fitted with thumb screws held the boards in place and thus when tightened prevented the jars from leaking. The air was filtered through cotton before it reached the cultures. Several times during the experiment the cultures subjected to dry air were moistened with a few cubic centimeters of water. It was found that those cultures were nearly dried out at these times.

No pycnidia were formed with peas, rice, or glucose agar under any of the conditions. Other cultures showed the pycnidia in the same relative proportions for the various conditions of aeration. The record for corn broth may be cited as typical.

TABLE XIII.—*Effect of humidity. Test with corn broth under bell jars*

[Time, 30 days]

Medium.	Number of pycnidia.					Growth.				
	Un-aer-ated.	Wet.	Mostly wet.	Mostly dry	Dry.	Un-aer-ated.	Wet.	Mostly wet.	Mostly dry.	Dry.
Corn broth. . . . .	o	o	o	+	+++	+++	++++	++++	+++	++

Pycnidia had been formed for some time before the reading was made. The aeration was continued, and a month later another reading was made. At this time all the cultures except peas, rice, and glucose agar showed pycnidia, irrespective of the air condition, with the exception of the series left as a check. This series, left in a battery jar, covered with a cotton pad and a glass plate of the same size as the jar, made good growth, but in no case did pycnidia occur.

We have in this experiment results which indicate that at most the effect of moist air is to delay pycnidium formation. Whether this effect is due to decrease in transpiration or to nutrition conditions, either of the substratum or of the aerial mycelium, brought about by the excess of water in the air or condensed upon the hyphæ is not known, but it seems likely that the water relation is the most potent one, since with such efficient aeration the transpiration must be considerable in all cases. The previous experiments indicated that absence of transpiration was not directly inhibiting to pycnidium formation with cultures which were under conditions of scanty nutrition. The last experiment reiterates that conclusion, but indicates that the humidity may serve to delay fruit-body formation. The effect of moist air in delaying but

not suppressing pycnidium formation is always associated with increased aerial growth. When it is recalled that with rich media the pycnidia are commonly formed in the aerial mycelium, this opposed condition may be significant. Further discussion of this behavior is given at another place (page 741).

In conclusion, it may be pointed out that transpiration, or, better, low air humidity, is a factor of only secondary or contributing influence in fruit-body formation for this fungus, and in no sense is a positive determining factor like light or aeration.

#### PHYSICOCHEMICAL FACTORS

##### REACTION OF THE SUBSTRATUM

The acid or alkaline reaction of nearly all biological fluids—the blood, milk, sea water, cell sap—varies but slightly from neutral. It is commonly said that fungi grow best under slightly alkaline conditions. Many organisms show great tolerance to either alkalinity or acidity, but the organism here investigated showed a comparatively narrow range, and its optimum point was not that of the great group of fungi, but much more like the optimum for bacteria.

The following experiment with filter-paper cones and with Raulin solution shows something of the limits of growth and reproduction for this organism. The acidity or alkalinity<sup>1</sup> indicated in the table was obtained by the addition of either normal potassium hydroxid or hydrochloric acid (potassium hydroxid in case of the Raulin solution, since it was acid at the outset).

TABLE XIV.—*Effect of acidity and alkalinity: Test with Raulin solution and filter paper*

[Time, 1 month]

Reaction.	Raulin solution		Filter paper.	
	Number of pycnidia.	Growth.	Number of pycnidia.	Growth.
—10.....	.....	Contaminated.....	.....	None.
—5.....	.....	None.....	.....	None.
0.....	+	Strong.....	20	Scant.
+5.....	++	Strong.....	20	Scant.
+15.....	o	Fair.....	.....	None.
+28.....	o	Fair.....	.....	None.

This experiment showed the strict relation of this organism to the chemical reaction, both as to growth and as to reproduction, and, as usual, the growth limits were wider than the limits of reproduction. The experiment also revealed why Raulin's solution had previously

<sup>1</sup> Computed in terms of cubic centimeters of normal hydrochloric acid or potassium hydroxid in a liter by titrating 5 c. c. with *N/20* standards, phenolphthalein as indicator.

given growth but no fruiting bodies. Once this relation of the organism to acid and alkali was known, previous experiments could be reviewed in the light of it and the behavior of certain chemicals explained.

Ten c. c. of a 5 per cent gum-arabic solution was autoclaved in a series of preparation dishes. The solution received sterile chemicals to give concentration as shown in the table and was inoculated with a spore suspension.

TABLE XV.—*Effect of acidity and alkalinity: Test with various chemicals*

[Time, 1 month]

Chemicals.	Concentration.	Reaction.	Number of pycnidia.	Growth.
Gum-arabic solution plus—				
Potassium acid phosphate.....	M/200.....	+	++	++
Potassium acid phosphate.....	M/200 each	+	+++	+++
+ Sodium acid phosphate.....				
Sodium acid phosphate.....	M/200.....	+	++	+++
Dibasic potassium phosphate.....	M/200.....	—	o	+++
Check.....		±	+	++
Sodium hydroxid.....		—5	o	+

This experiment, if it be permitted to draw conclusions by comparison of salts with a similar anion or cation, indicated that the specific effects in pycnidium formation were not due to any specific ion, for if potassium were the influential ion, then we should get no effects with the similar sodium salt. More conclusive still was the effect of the dipotassium phosphate as contrasted with the dihydrogen salt. Here the same ions were concerned, but in different proportions. The experiment shows the extreme sensitiveness of this organism to alkalinity, since a reaction of  $-5$  was sufficient to cause absence of pycnidia.

A study of the reaction of some of the common media, as given in Table XXV, shows how reaction controls not only reproduction, but growth as well. Of the complex media tried the most favorable for pycnidium production was a couple of corn grains autoclaved in 10 c. c. of water. Aside from the nutrition relation, which will be discussed later, the acid reaction is largely responsible for the excellence of this medium; but the time when this reaction is most effective is at the period when growth has covered the medium, not the mere reaction at the start. Corn broth shows at the start an acidity of  $+8$ , and after a month the reaction is still acid,  $+5$ . As is seen from Table XIV, this is a favorable condition for pycnidium production. Pea solution at the beginning of a period of culture showed an acid reaction of  $+8$ , while oats showed at the start a reaction of  $+5$ . The latter showed after a month a reaction approximately neutral. It will be seen from Table XXV that oats were a correspondingly poorer medium than corn. Pea broth, on the other hand, showed a reversal of condition, and after a month

titrated —8. The culture grew vigorously for a week or two, formed a mat and some aerial mycelium, then the gradual checking of the growth occurred. The culture ceased producing aerial mycelium and the mat became half submerged. Soon all growth ceased and the culture grew but indifferently or not at all when transplanted.

If old pea-broth cultures were acidified to approximately +5 with potassium acid phosphate, tartaric acid, or hydrochloric acid, growth started again and pycnidium production took place upon the dense mat.

Other media showed similar changes in either acid or alkaline reaction, and, as a rule, it may be said that media with a proportion of protein lower than the carbohydrate proportion show after a period of growth an acid reaction (Wehmer, 1891). With media high in protein the reaction becomes alkaline (Nägeli, 1880).

The consideration of the acidity or alkalinity of substrata at the start and at the close of a period of culture leads naturally to a consideration of autointoxication. This is especially appropriate in this case, since the autointoxication effects observed were due to harmful reactions produced by the by-products of metabolism. These by-products were not of the complex type commonly thought of in connection with the term autointoxication, but were mostly the simple and well-known end products of carbon and nitrogen dissimilation. The injurious effects were produced to a large extent by the acidity or alkalinity engendered, and the same effects could be artificially produced in a favorable medium by mere change of reaction.

Depending upon the excess of carbohydrate or protein, as has been said, the reaction of the substratum became either acid or alkaline. In the case of excess of carbohydrate, oxalic acid is formed by this organism, and in old cultures of corn, oats, or prune-juice agar crystals of calcium oxalate were often found. In the case of protein excess, as was demonstrated for old pea-broth cultures, the medium contained an excess of ammonia. This ammonia could be detected by boiling the liquid from such old cultures and testing the fumes with a strip of wet, red litmus paper.

In a solution where the carbohydrates and protein constituents are present in a proper ratio, these by-products of metabolism neutralize each other. Corn broth is a notable example of this type of medium, for in it the by-products, even after two months, are not potent enough to interfere with reproduction.

The action of these autointoxication products in the substratum is further illustrated by the common experience met with in transferring from old cultures of this organism. In old agar cultures of various sorts the mycelium was found dead when it was submerged in the substratum, although the aerial mycelium remained alive for more than a year.

We have, therefore, in autointoxication a phase of the major factor, acid or alkaline reaction, and while definite harmful bodies of a protein or amid type are known for organisms and may have been present here, we have in the end products of protein and carbohydrate dissimilation harmful constituents whose influence may be to limit either growth or reproduction.

#### CHEMICAL FACTORS

##### QUANTITY OF FOOD

The quantity, rather than the quality, of the food needed for this organism can more conveniently be considered at the outset. As was stated at the beginning of the experimental work, there is a certain minimum for growth and also for reproduction. Naturally, reactions taking place at the base level of nutrition are sharper and less obscured than those taking place where food is in abundance and the factors of reaction, autointoxication, etc., have greater and greater influence. For this reason, once the capacity of this organism to grow and reproduce upon material almost devoid of nutrients was recognized, many of the experiments with other factors have been performed with the food supply reduced to a low level.

This power to grow upon simple stuffs and with them in extremely high dilution naturally led to the question of the minimum essential. Growth and reproduction in distilled water has already been mentioned. The distilled water used in the first experiments was the ordinary distilled water of the laboratory. The glassware used was "resistance," cleaned as described. The test tubes were plugged with cotton, and a few motes of cotton could be seen upon the surface of the water after inoculation. Inoculation was made as described with a spore suspension. The number of colonies which resulted from inoculation with similar-sized drops of this suspension in Raulin solution was from 5 to 20. These details show that a very small amount of organic stuff was introduced from the inoculum. After three or four weeks a white or gray filmlike mycelium could be seen, either attached to the glass or floating near the bottom of the test tube. After a month or, at times, two months 2 to 5 pycnidia were produced under the water.

It is difficult to understand where the carbon and nitrogen used by the fungus came from. The minerals might be accounted for more or less satisfactorily by assuming that they came from the glass, which is slightly soluble. For the organic stuffs we have a few possibilities. The nitrogen may have come from ammonia in the air, and the carbon from the small bits of cotton dropped from the cotton plug. It is more than likely that the distilled water carried some oily volatile material, which, while not strongly influencing conductivity, gave a suitable foodstuff for the fungus. Or we have the possibility, first pointed out by Elfving (1890), that organisms may be fed by small quantities of volatile sub-



stances which are absorbed from laboratory air by the water (Beijerinck and Van Delden, 1903). Be the source of this food supply what it may, I was interested to find if all distilled water, even the purest, had enough food supply or absorbed enough to support both growth and reproduction.

Conductivity water<sup>1</sup> of a value  $3.03 \times 10^{-6}$  was used as in the preceding experiment, with, however, the following improvements in the method. Jena glass test tubes were used throughout. The test tubes were plugged with long-fiber absorbent cotton, and the preliminary dry sterilization, which has a tendency to make the fibers brittle, was omitted. Inoculation was made with one drop of a filtered spore suspension which had about 25 to 50 spores to the drop. The pycnidium which furnished these spores was growing in aerial mycelium, so none of the old substratum was brought over. At all events, material brought with the spores was diluted nearly 200 times. After two months slight growth was evident as faint submerged wisps or skeins. The growth was less than a tenth as strong as that produced in ordinary distilled water. No pycnidia were formed.

This experiment indicates that in the soluble glass and in the character of the distilled water we have the important sources of the food supply. The mites of cotton were practically eliminated in the last experiment. It might be thought that the nutrition in this case was as good as the preceding—assuming the food supply to come from volatile chemicals—and that the poor growth of mycelium and the failure to reproduce was due to the toxicity of the conductivity water. But the toxicity of ordinary distilled water is generally admitted to be greater than the toxicity of conductivity water. Moreover, this organism has never shown any effects which might be attributed to toxic substances in the water. In the recent experiments on the toxicity of distilled water with other plants the nutrition phase has been neglected, since the conclusions have been drawn from tests with the well-nourished roots of seedlings. In the experiments here reported, the food supply carried in the plants is that which is within a few spores barely visible with the high power of the microscope. It is difficult therefore to attribute the effects to anything but the scantiness of nutrition.

The conclusion, therefore, is drawn that while growth and reproduction can take place with the meager food supply of ordinary distilled water in "resistance" glass, the limit of reproduction is reached with conductivity water and Jena glass, but the limit of growth is still lower.

This same relation to nutrition was shown with the following experiment with filter paper. It had been determined in many previous experiments that this organism could grow and reproduce upon filter paper and distilled water. Tests with tap water, distilled water, and conductivity water indicated that the material used for growth and

<sup>1</sup> I am indebted to Dr. R. P. Hibbard, of the Michigan Agricultural College, for the conductivity water. The measurements of resistance were also made by him.

reproduction came largely from filter paper. Although filter paper is said to be the purest form of cellulose obtainable, Schwalbe (1910-11, p. 600) states that appreciable amounts of oxycellulose and hydrate-cellulose are present. Since filter paper is known to have some ash, a preliminary experiment was performed to find if this ash served, in part at least, as a source of food. A pair of culture dishes was prepared with a filter-paper cone in each. Ten c. c. of ordinary distilled water were added. To each of two other dishes with a similar amount of water, the ash from a filter cone was added. These dishes were autoclaved. Inoculations were made with spores. After three weeks the results shown in Table XVI were obtained.

TABLE XVI.—*Effect of quantity of food: Test with filter paper and the ash from filter paper*

[Time, 3 weeks]

Medium.	Pycnidia.	Growth.
10 c. c. distilled water, plus filter cone.....	+	Good.
10 c. c. distilled water, plus ash.....	—	Scanty.

The better growth and the pycnidial production on the filter paper, as opposed to the results with ash, indicate that the influential stuffs are not those from the ash. It may be remarked that the readings were taken early enough to avoid complications due to the slow pycnidium formation in distilled water. The effect of ash having been shown to be negligible, the main experiment was set up. Five sheets of filter paper (S. & S. 595) about 15 cm. across were autoclaved in 500 c. c. of conductivity water in a Jena flask. This furnished a stock solution, which was diluted with conductivity water by means of pipettes and graduates, which were carefully rinsed before and during the operations. The dilutions were prepared in Jena beakers, but were eventually put in 10 c. c. quantities in a number of Jena test tubes. These were autoclaved and inoculated with a spore suspension. This experiment was done in duplicate with each of the strains of the fungus, with the results shown in Table XVII.

TABLE XVII.—*Effect of quantity of food: Test with filter-paper broth*

[Time, 2 months]

Medium.	Pycnidia.	Growth.
Filter-paper broth:		
1/1.....	+ (3)	Fair, easily seen.
1/100.....	+ (1)	Fair, easily seen.
1/1,000.....	+ (1)	Scant, barely visible.
1/10,000.....	—	Scant, barely visible.
Conductivity-water check.....	—	Scant, barely visible.

The experiment shows that the ordinary high-grade filter paper, when autoclaved with water of high purity, yields sufficient nutriment for growth and reproduction of this organism. A dilution of 1/100 is still sufficient for pycnidium production, but at 1/1,000 we have reached the limit of food supply sufficient for pycnidium production. Growth, as usual, takes place at greater limits than reproduction.

This experiment gives conclusive evidence that the toxic substances of distilled water do not affect this organism. We may now conclude that we have been working nearer and nearer the limits of growth and reproduction. The amount of material required is evidently extremely minute. It is in the imponderable mass of stuff, somewhere between distilled water and conductivity water, or in that bulk of stuff lying between 1/1,000 and 1/10,000 dilution of a filter-paper broth.

Having now some conception of the extremely low limits of concentration at which growth may take place, we may now consider the growth and reproduction relations at higher concentrations.

The experiments already reported give a mass of details as to growth, at various concentrations, but no conclusions from these isolated cases are justified, because the reaction is so masked by other relations.

The following experiments allow a comparison of some nutrient solutions at various concentrations. The solutions chosen were those which did not become toxic with the continued growth of the organism. In one experiment 200 grains of corn were autoclaved in 1 liter of tap water. This solution was concentrated to approximately 100 c. c. by boiling in a beaker. It was, therefore, approximately 10 times the strength of ordinary corn broth. The strong solution was also diluted as shown in the table. Cultures were made as usual and were inoculated with a spore suspension. The results are shown in Table XVIII.

TABLE XVIII.—*Effect of quantity of food. Test with corn solution*  
[Time, 1 month]

Concentration.	Pycnidia	Growth.	Remarks.
10X.. . . . .	—	+++++	White.
5X..... . . . .	—	+++++	White.
1X. . . . .	+++	++	Blackened.
1/10X.. . . . .	+	+	Blackened.

In this experiment it is seen that the organism, after a month, produced fruiting bodies only in the lower concentrations, but the growth was strong in the higher concentrations. The growth in the weaker concentrations had increased but slightly after the first two weeks. We may conclude then that a food supply which allows a fair growth and then becomes exhausted is most favorable for pycnidium formation.

The following experiment with synthetic media was performed. The combination described upon page 752 was made up at 25 times the usual

concentration. This was diluted as shown in Table XIX, and cultures were made as in the preceding experiment.

TABLE XIX.—*Effect of quantity of food. Test with synthetic solution*

[After 1 month]

Dilution.	Pycnidia.	Growth.	Remarks.
25X . . .	—	o	White or pinkish. Black mat formed. Slightly less growth than above, black mat. Slightly less growth than in 2X. Abundant evidence of pycnidia starting.
10X . . .	—	o	
5X . . .	o	++++	
2X . . . . .	o	+++	
1X . . . . .	Many.	+++	
1/2X . . .	Many immature.	++	Growth weak. Pycnidia extremely minute. Mycelium scanty.
1/5X . . .	10	+	
1/10X . . .	5	+	

The experience with this solution shows that doubling the concentration of a favorable culture solution increased growth, and was sufficient to inhibit completely pycnidium formation. A solution diluted one-half gave promise of many pycnidia—more than in the 1X concentration—but the pycnidia were slow in forming. In the extremely low concentration growth was scant and a small amount of pycnidium production took place. The experiment leads to the same conclusions as the preceding experiment—i. e., that a limited food supply is essential to fruit-body formation, and the optimum concentration is one which gives a comparatively large mycelial growth before the exhaustion takes place.

The teaching of this experiment would place the limit of concentration of a sugar at  $M/100$ . We have, however, a great body of experiments already outlined in which pycnidium production took place with a sugar concentration considerably higher. For instance, in Table X pycnidia are reported for Raulin's solution (cane sugar  $M/7$ ) when a calcium salt was added. Or, considering the experiments with corn grains, these seem to present a contradiction when it is noted that the pycnidia were first formed on the corn grain with its rich food supply. Similarly, the various laboratory media—such as prune-juice agar, parsnips, and carrots—all are rich in carbohydrates; yet these are reported as allowing pycnidium production.

In these rich solutions, however, an extremely abundant aerial mycelium is produced, and as the medium begins to dry the pycnidia are produced in the aerial strands, but never upon the medium itself. In a few cases a dense mat formed over the agar, and this effectively walled off the new food supply. On only one laboratory medium—corn-meal agar (Shear and Wood, 1913)—were the pycnidia produced directly upon the agar. It is noteworthy that with this medium the mycelium production is scant. In the case of corn grains the pycnidium production does not take place until the corn grain is dried somewhat, and this, coupled with

the fact that the corn grain is not extremely soluble, accounts very well for the appearance here. Instead of the corn grain furnishing nutrition, the corn grain soon becomes the location where food supply is soonest exhausted. In this behavior upon drying, we may also find the explanation of the behavior of the wet and dry bell jars reported in Table XIII. The behavior of the 1 × and ½ × concentrations of the synthetic medium may be considered in this connection. It seems that in this case we have a similar factor to deal with. The mycelium in these concentrations grows at the top of the solutions, a trifle submerged in the case of the weaker solution. The stronger mycelial growth in the higher concentration leads to the formation of a thicker surface film in it than in this weaker one, and the film starts much sooner. The pycnidia are produced upon this surface film, which, no doubt, in some ways interferes with the utilization of the food supply.

From this it would seem that the limiting concentration suggested— $M/100$  for sugar—instead of being too low is doubtless too high, and the production of pycnidia at this concentration, at the period stated, is brought about by the other factors, which lead to an even greater reduction of the available concentration.

When we consider the action of this aerial life of the mycelium in fostering reproduction, we find that our knowledge of the transfer of materials in mycelium is extremely limited. It, however, seems very likely that with the increase in concentration in the medium below and the drying of the threads, the diffusion of foodstuffs to the aerial parts is interfered with.

#### QUALITY OF FOOD

**MINERALS.**—The work with the quantity of foodstuffs just outlined indicates the extreme difficulty of determining what minerals are essential for growth. This sensitiveness to extremely small amounts, which doubtless is paralleled by other organisms, makes experimentation with ordinary methods or ordinary chemicals unreliable. The problem of determining the necessary mineral elements for this fungus would be impossible with our present technic.

An attempt was made to find the effect of certain chemicals when they were added to various nutrient solutions. Although many experiments were performed, the results were so masked or influenced by the constituents of the medium that no conclusions could be drawn. Notable influences which have been explained as other than nutrition effects have been obtained with acid phosphates and with calcium compounds.

The behavior of one chemical, magnesium sulphate ( $MgSO_4$ ), is worthy of record. Since Molisch's accurate work (1894), this substance has generally been regarded as essential in fungous cultures. The following experiment suggests that the chemical may have a profound effect upon fructification. Two preparation dishes each received 10 c. c. of a solution

containing magnesium sulphate in  $M/33$  concentration. Conductivity water was used. Inoculation was made with a drop of spore suspension. After one month many (more than 50) pycnidia were found in the loose submerged mycelium.

As a mineral base for nutrient solutions, monobasic potassium phosphate and magnesium sulphate, along with other chemicals, were frequently employed. The net result of numerous cultures made in the attempt to find some hint of the value of this or that mineral was the conclusion that cultures with these two constituents alone, with a suitable nitrogen and carbon supply, gave as good results as more complex combinations.

This solution of mineral salts contains the bulk of the elements generally considered essential for fungus growth. Carbon and nitrogen need to be added to secure the complete nutrient, but iron can be neglected, since it is such an unavoidable impurity in chemicals and is usually present as a constituent of the glassware. Beijerinck (Samkow, 1903) had used a similar solution as a culture medium for bacteria.<sup>1</sup>

Because of the extremely small amounts of minerals found necessary for growth and reproduction in this form, I modified the formula by cutting down the concentration of the various components. Since the solutions were to be used in comparative work, the chemicals were added on a molecular-weight basis. At the time of the first experimentation it was thought that the reaction should be approximately neutral, and accordingly molecularly equivalent weights of potassium acid phosphate and sodium carbonate were employed. Similarly, through dependence upon relations of other plants, it was thought that magnesium sulphate might be slightly toxic, and it was used at a lower concentration than either of the other two minerals. The solution thus devised for preliminary experiments contained sodium carbonate and potassium acid phosphate as  $M/100$  and magnesium sulphate as  $M/500$ . Subsequent experiment showed that the carbonate could well be omitted and the magnesium sulphate increased from fivefold to tenfold.

The other combinations were used for comparison with this mineral base. The mineral constituents of Raulin solution and those of Dox solution were tried, and while either were suitable, neither had any advantage over this modified Beijerinck solution; on the contrary, they were much more complex and contained the mineral elements in excess of the needs of this fungus.

**CARBON SUPPLY.**—The carbohydrates form the common source of carbon for fungi. Other classes of compounds, as pointed out by Nägeli (1880) and Wehmer (1891), may be utilized. For this organism, as indicated in Table XXIII, other classes of compounds—but of alcoholic

<sup>1</sup> Samkow used the following base with a great variety of organic compounds: Potassium acid phosphate, 2 gm.; sodium carbonate, 2.5 gm.; magnesium sulphate, 0.4 gm.; water, 1 liter.

structure—may be utilized as a carbon source (malic acid and glycerol). As is well known, various plants possess widely varying amounts of sugars, and the sugars and other carbohydrates differ markedly in kind. The specific effects of certain vegetable media have been attributed by many to the specific action of the type of carbohydrate furnished. Roux and Linossier (1890), as a result of their work with the fungus *Dematium albicans* Laurent, announced as a general biological law that with an increase in the molecular weight of the carbohydrates the complexity of the growth form of the fungus increased. With certain sugars, such as glucose in a 1 per cent solution, these investigators obtained only yeastlike growth, but with a biose, such as maltose, they obtained strong mycelium and conidia production. Recently Hiekel (1906), repeating the work of Roux and Linossier, but with 10 per cent sugar solutions, accepted the conclusions of the French investigators within certain limits. A priori, it is very difficult to see why two sugars, such as glucose and maltose, should differ in specific effects, since the latter, when hydrolyzed, yields only the former.

Very early in the investigation tests were made with the common sugars to find whether there was a specific effect on fruit-body formation due to the various sugars. In these tests the sugars used were used as weight-normal solutions; hence, the effects secured were not obscured by concentration differences. The various sugars were added from a sterile stock *M/1* solution to 10 c. c. of the autoclaved nutrient solutions, as indicated in the table. Glass preparation dishes were used, and all were placed in strong diffuse light. Inoculations were made with spore suspension in the usual manner. The tests were done in duplicate. Table XX shows the average of conditions.

TABLE XX.—Effect of quality of food: Test with sugars

Sugar.	Pea broth.			Oat broth.			Tap water and filter.		
	Sugar concentration.	Pycnidia.	Growth.	Sugar concentration.	Pycnidia.	Growth.	Sugar concentration.	Pycnidia.	Growth.
Saccharose....	M/10	o	+++	M/10	o	+++	.....	.....	.....
Do.....	M/20	o	++++	M/20	o	+++	.....	.....	.....
Do.....	M/50	o	+++	M/50	o	+++	M/50	o	++
Dextrose.....	M/10	o	.....	M/10	o	+++	.....	.....	.....
Do.....	M/20	o	++++	M/20	o	+++	.....	.....	.....
Do.....	M/50	o	+++	M/50	o	+++	M/50	o	++
Levulose.....	M/10	o	+++	.....	.....	.....	.....	.....	.....
Do.....	M/20	o	++++	.....	.....	.....	.....	.....	.....
Do.....	M/50	o	+++	.....	.....	.....	M/50	+	+
Maltose.....	M/10	o	++++	.....	.....	.....	.....	(1 or 2)	.....
Do.....	M/20	o	++++	.....	.....	.....	.....	.....	.....
Do.....	M/50	o	+++	.....	.....	.....	M/50	o	++
Check.....	.....	o	++	.....	+	++	.....	+	+

It will be noticed that in nearly every case, even in low concentration of sugar, there was an increased growth following the addition of sugar. Filter paper and oat broth, which normally produce pycnidia, gave strong growth with saccharose, dextrose, and maltose, but no pycnidia. In the case of levulose *M/50*, the growth was not greatly increased, and one or two pycnidia appeared. This number is much less than the normal for filter paper alone. We may conclude that these sugars exert a repressing influence on pycnidium production, and at the same time augment vegetative growth. How this is brought about is difficult to explain; but in some way the ratio of the constituents was so altered that the limits for reproduction of some factor—e. g., reaction—or of some group of factors was exceeded.

A more comprehensive experiment was performed in which a large number of carbohydrates was tested. Equal parts of the minerals of Raulin's solution in  $2 \times$  concentration were added to various *M/10* sugar solutions and to 2 per cent solutions of the polyoses whose molecular weight is not known. Each combination was set up in four capsules, using 10 c. c. per dish. The media were steamed on three successive days and inoculated with a drop of spore suspension for each dish. Table XXI gives the result of this experiment.

TABLE XXI.—*Effect of quality of food: Test with carbohydrates*  
[Time, 2 months]

Carbohydrate.	Concentration.	Size of colonies.	Growth	
			Character.	Form of fructification.
		<i>Mm.</i>		
Xylose (pentose).....	<i>M/20</i> ...	3-4	Compact..	Oidia.
Maltose (disaccharose).....	<i>M/20</i> ..	3	Compact..	Oidia.
Glucose (monosaccharose).....	<i>M/20</i> ...	2	Compact.....	Oidia.
Mannose (monosaccharose).....	<i>M/20</i> ...	2	Compact.....	Oidia.
Galactose (monosaccharose).....	<i>M/20</i> .....	2	Compact.....	Oidia.
Levulose (monosaccharose).....	<i>M/20</i> .....	1-2	Compact.....	Oidia.
Arabinose (pentose).....	<i>M/20</i> ..	1-2	Compact.....	Oidia.
Sorbose (monosaccharose).....	<i>M/20</i> .....	(2½)	Floccose.....	Pycnidia.
Sucrose (disaccharose).....	<i>M/20</i> .....	½-1	Compact.....	Oidia.
Raffinose (polysaccharose).....	<i>M/20</i> .....	2-3	Floccose, very loose.	Mycelium.
Lichenin (polysaccharose).....	1 per cent.....		Loose mat, covering dish.	Secondary spores.
Dextrin (polysaccharose).....	1 per cent.....		Diffuse mat, covering dish.	Pycnidia.
Inulin (polysaccharose).....	1 per cent.....		.....do.....	Pycnidia.
Gum arabic (polysaccharose).....	1 per cent.....		.....do.....	Pycnidia.
Gum tragacanth (polysaccharose).....	1 per cent.....		.....do.....	Pycnidia.
Wheat starch (polysaccharose).....	1 per cent.....		No growth.....	
Lactose (disaccharose).....	<i>M/20</i> .....		No growth.....	
Erythrose (tetrose).....	<i>M/20</i> .....		No growth.....	



In the above table the sugars and other carbohydrates are arranged on the basis of vigor of vegetative growth. In the main the results of the former experiment are substantiated. The strongest growth took place with the highly soluble sugars, and the dishes were filled with small ball-like masses. The strongest growth was not associated with pycnidia production, but on the contrary was opposed to it. At first glance the law of Roux and Linossier (1890) seems operative, for pycnidia appeared in the carbohydrates, which are known to have extremely high molecular weights. But this superficial agreement is abundantly contradicted by the first part of the list. Without regard to molecular weight, these sugars gave approximately the same growth form, and the variation in amount of growth was not striking. It will be noted that these sugars are highly soluble, while those toward the bottom of the list are almost insoluble. In the one case every bit of the foodstuff was available, while in the other only a slight amount of the carbohydrate was open to appropriation. The preceding experiment with filter paper and sugars proved that, where the scant available carbohydrate of filter paper allowed pycnidia production, the addition of sugars destroyed the balance between growth and reproduction, and only growth took place. The same general relations exist between the members in this table as existed in the former experiment. It is worthy of note that Roux and Linossier (1890) and later Hiekel (1906) drew their conclusions from carbohydrates such as the first seven. We can find in their method of work the source of their error. Their solutions were made up on a percentage basis, and where they drew a conclusion that a complex sugar like maltose in 1 per cent solution gave a more complex growth than a 1 per cent glucose solution, because of the difference in molecular weight, they were in reality comparing  $M/36$  and  $M/18$  solutions, and their conclusion really applies to concentration. They had previously shown that a low concentration would call out more complex growth forms.

The cause of the variation in growth among the various sugars is not known. A great many factors undoubtedly enter. Nearly all the sugars used were split in approximately the same way by the various specific enzymes of the organism. Differences in absorption rates, in rapidity of enzymotic action, etc., may enter and be responsible for the differences in growth here recorded. It may further be remarked that although the sugars used were of the highest purity they vary in their relative freedom from contamination, owing to difficulties in separation and purification. The colloidal carbohydrates undoubtedly carry a mass of adsorbed material, while in the others, traces of calcium, nitrogenous material, etc., may be present. It is not unusual to find a minute gummy scum on freshly prepared maltose solution.

Certain other interesting points are to be found in the table. The production of the growth form called "oidia"—multiseptate, heavy-walled hyphae resembling *Dematium* or at times *Monilia*—were constantly

found in the highly soluble sugars. Such growth forms have commonly been recognized as a reaction to high osmotic pressure. Ternetz (1900) has obtained these in acid solutions. But such growth forms have occurred with this fungus in distilled water and on filter paper, and no doubt this growth form, instead of being a specific reaction to concentration, is one induced by a number of unfavorable conditions.

The action of sorbose has been disregarded, because this sugar is broken down by heat. The failure to obtain growth with lactose and erythrose is not without parallel in the literature. The action of wheat starch is peculiar, in view of the previous successful use of potato starch (Table X).

The action of lichenin is of great interest. This carbohydrate is a dextrin-like compound, almost insoluble in cold water and forming a gummy mass in hot water. In the turbid solution of this chemical the fungus produced a great number of secondary spores, evidently hyphomycetous. These spores were of approximately the same size and shape as the ordinary spores of this fungus. The exact method of their production was not determined. Mounts of material gave only straight mycelial threads and great numbers of detached spores. Dilution plates poured from the culture dishes teeming with these spores gave no other organism than the one under investigation. The colonies appeared in the plates in such abundance as to leave no doubt concerning the relation of these colonies to the secondary spores.

The experiments with carbohydrates may now be summarized. Nearly all carbohydrates tried served as a source for carbon. The general effect of adding sugars even in so low a concentration as  $M/50$  was to stimulate vegetative growth greatly, but this stimulated growth was accompanied by a pronounced repression of pycnidium formation. In an experiment with  $M/20$  solutions a strong mycelial growth was obtained, accompanied by oidia-like bodies, but fructification was absent. With slightly soluble carbohydrates, in which the actual amount of available soluble material was always limited, vegetative growth was weaker and pycnidium production was a general rule. A comparison of these highly soluble and slightly soluble carbohydrates indicates that the differences in growth form are connected with the amount of food supply rather than with the specific nature of the sugar. This position is reinforced when we consider that the hydrolysis of inulin, gum arabic, etc., yields exactly those sugars which, when tested in  $M/20$  concentration, gave no pycnidia. In view of this comparison the earlier conclusion of Roux and Linossier (1890) seems untenable, and a more plausible explanation of the differences of growth form obtained seems to be found in the concentration relations.

This matter of carbohydrate supply has obviously a marked influence upon the problem of the organic media for laboratory use.

**NITROGEN SUPPLY.**—That the organism was influenced by the kind and amount of the nitrogen supplied seemed evident from the results of experiments with standard media, such as beef broth and beef agar, as well as the results already reported for pea broth.

A number of preliminary experiments of the same type as those reported under carbohydrates were performed at the same time, and these indicated that the various nitrates influenced pycnidium formation. But these results were not altogether consistent. The following experiment (see Table XXII) with filter paper and tap water plus various chemicals, and the similar series in which distilled water was used, may be cited as typical.

TABLE XXII.—*Effect of quality of food: Test with various nitrates*  
[Time, 1 month]

Chemical.	Present as—	Distilled water.		Tap water.	
		Pycnidia.	Growth.	Pycnidia.	Growth.
Calcium nitrate.....	M/100 .....	20-30	++	20-50	+++
Potassium nitrate.....	M/100.....	50-100	+++	50-100	+++
Calcium acid phosphate ( $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ) + calcium nitrate.	M/200, M/100	30-50	+++	100+	++
Potassium acid phosphate + potassium nitrate.	M/100 each	.....	+++	100+	+
Filter paper.....	Check.....	9-20	+	7-12	+

From this experiment it could not be determined beyond question that the nitrate ion was the potent factor in this increase in pycnidia formation, but the corresponding behavior of both the calcium and the potassium nitrate indicated that this was extremely likely. The increase in pycnidium production upon the addition of both a phosphate and a nitrate to this carbohydrate medium is significant.

Since the nature of the carbon assimilation might greatly influence the nitrogen assimilation, experiments with these two compounds can hardly be separated. In the following experiment an attempt was made to test various classes of carbon-furnishing compounds with various nitrogen sources. In this experiment the mineral solution mentioned in the preceding section was used. The stock solution contained monobasic potassium phosphate as M/100, sodium carbonate as M/100, and magnesium sulphate as M/500. To different portions of this, malic acid, glycerol, and maltose were added, respectively, so that each chemical was present at M/100 concentration. A fourth series was prepared as a check, and in this cones of filter paper furnished the carbon supply (S. & S. 605). The various solutions were put into series of preparation dishes, 5 c. c. per dish. To these dishes the nitrogen compounds to be tested were added from a clean pipette 1 drop (1/20 c. c.) of the proper solution (stock solutions were made up M/50, except peptone, which was 2 per cent) to

each dish. The various combinations employed, and the dilutions present in the culture, are indicated in Table XXIII. In every instance the concentration given shows the amount of the chemical that was present in the culture. The experiment was done in quadruplicate.

TABLE XXIII.—*Effect of quality of food: Test with nitrogen and carbon compounds*

Stock solution of minerals plus—		Number of pycnidia.	Growth.
Carbon.	Nitrogen		
Malic acid, <i>M</i> /100.....	Peptone, 0.02 per cent.....	100	Scant.
Glycerol, <i>M</i> /100.....		60	Strong.
Maltose, <i>M</i> /100.....		0	Strong.
Filter paper.....		.....	No growth.
Malic acid, <i>M</i> /100.....	Asparagin, <i>M</i> /100.....	.....	None.
Glycerol, <i>M</i> /100.....		5	Scant.
Maltose, <i>M</i> /100.....		100+	Strong.
Filter paper.....		.....	None.
Malic acid, <i>M</i> /100.....	Leucin, <i>M</i> /600.....	5	Scant.
Glycerol, <i>M</i> /100.....		0	Scant.
Maltose, <i>M</i> /100.....		25-50	Strong.
Filter paper.....		.....	None.
Malic acid, <i>M</i> /100.....	Potassium nitrate, <i>M</i> /500....	.....	None.
Glycerol, <i>M</i> /100.....		2-7	None.
Maltose, <i>M</i> /100.....		50	Fair.
Filter paper.....		0	Scant.
Malic acid, <i>M</i> /100 ..	.....	10-20	Fair.
Glycerol, <i>M</i> /100.....	.....	0	Scant.
Maltose, <i>M</i> /100.....	.....	0	Fair.
Filter paper.....	.....	10-15	Fair.
Peptone.....	.....	1-5	Scant.
Asparagin, <i>M</i> /500.....	.....	0	Scant.
Leucin, <i>M</i> /600.....	.....	0	Scant.
Potassium nitrate, <i>M</i> /500..	.....	0	Scant.

This experiment shows that nitrogen, as previously shown for carbon, may be taken from widely different classes of compounds. The availability of any particular nitrogen compound is largely determined by the associated carbon compound. For instance, peptone, which carries available carbon, gave a large number of pycnidia with malic acid, but none with maltose. Asparagin, which gives the best growth and the greatest number of pycnidia with maltose, gave no pycnidia with malic acid. Glycerol, which seems on the whole to be a poor carbon source, gave with peptone strong pycnidium production, but with other nitrogen compounds behaved indifferently. As a further complication, peptone is able to serve both as nitrogen and as carbon source. Leucin gave poor growth with all carbon compounds except maltose, and a comparison of its behavior with that of asparagin, which is a compound of the same class, is interesting.

The experiment shows in a striking way how unlimited the possible combinations of nutrients may be. The marvelous thing is the absolute regularity of the product, regardless of this or that varied food supply. Growth that morphologically could not be distinguished arose from a protein or a mineral nitrate. Pycnidia were produced from these widely divergent compounds, with carbon compounds equally separated, and in these were billions of spores which did not differ in a manner permitting measurement, each a potentiality which could repeat indefinitely under these conditions the same reaction.

From this experiment we may pick a combination which is favorable for growth, but which also gives an abundance of pycnidia. For further experiments the combination of minerals with maltose and asparagin was chosen. The steps, more or less logical, which lead to the development of this synthetic culture solution may be reviewed. Experiment had shown that the essential mineral elements necessary for the growth and development of this fungus were contained in two mineral salts. Experiments in which these are added to various nutrient solutions give a hint as to the value and the concentration suitable. Eventually a compound was selected which gave the mineral salts which needed to be supplied and in addition had a chemical which could be used to make the reaction less acid, as desired. Previous work had shown that the organism could grow and produce pycnidia on extremely limited amounts of minerals, so the amounts taken were extremely small—far smaller than the ordinary formulas call for. In choosing the carbohydrate, wide choice was possible, since so many allowed good growth. Maltose was selected for use in the experiment just reported, because, next to xylose, it had given the best growth. The use of xylose was not advisable because of its high cost, but care was taken to use maltose in small amount, so that the effect found in the experiment reported in Table XX would not be repeated. Accordingly, *M/100* concentration was provisionally chosen. The device for deciding upon the nitrogen source has been detailed in the preceding experiment. The low concentration of nitrogen was chosen to avoid such toxic conditions as were found in the pea broth. In passing, it may be said that an attempt was made to secure approximately the ratio of carbon to nitrogen that exists in the corn broth, which had been found extremely favorable to the organism.

Different concentrations of the separate constituents of this nutrient solution were further tested, with extremely interesting results. The device used was to vary the concentration of one constituent while holding the others constant. It was thought that in this way approximately the optima for all the constituents could be found.

The following experiment was performed with double-distilled water (slightly poorer than conductivity grade) and "non-sol" glass flasks. Dilutions were prepared as outlined in the table, and the culture media

steamed on three successive days. It was found that steaming instead of sterilization under pressure was very important. In a previous attempt the media were sterilized in the autoclave, and upon inoculation absolutely no growth took place. The experiment was done in quadruplicate with one strain. Inoculation was made with a spore suspension as before. The flasks were set in strong diffuse light near a window. Readings were made after a month. Five c. c. of water were added to each flask, and a second set of readings<sup>1</sup> were made after another month. The result of the experiment is shown in Table XXIV.

TABLE XXIV.—*Effect of quality of food: Test with synthetic solution in various combinations*

[Time, 2 months]

Medium.		Number of pycnidia.	Growth.		
Potassium acid phosphate, <i>M/100</i> .	} Plus asparagin . .	<i>M/50</i> . . .	0	±	
Sodium carbonate, <i>M/100</i> . . . . .		<i>M/100</i> . . .	0	±	
Maltose, <i>M/100</i> . . . . .		<i>M/500</i> . . .	50	++	
Magnesium sulphate, <i>M/500</i> . . . . .		<i>M/1,000</i> . . .	0	+	
		<i>M/5,000</i> . . .	0	+	
Potassium acid phosphate, <i>M/100</i> .	} Plus maltose . . . .	<i>M/10</i> . . . .	0	+++	
Sodium carbonate, <i>M/100</i> . . . . .		<i>M/50</i> . . . .	0	+++	
Magnesium sulphate, <i>M/500</i> . . . . .		<i>M/100</i> . . . .	50	++	
Asparagin, <i>M/500</i> . . . . .		<i>M/200</i> . . . .	0	+	
		<i>M/1,000</i> . . .	0	+	
Potassium acid phosphate, <i>M/100</i> .	} Plus magnesium sulphate.	<i>M/50</i> . . . .	<sup>a</sup> 13	++	
Sodium carbonate, <i>M/100</i> . . . . .		<i>M/100</i> . . . .	1	+++	
Maltose, <i>M/100</i> . . . . .		<i>M/500</i> . . . .	50	++	
Asparagin, <i>M/500</i> . . . . .		<i>M/1,000</i> . . .	0	++	
		<i>M/5,000</i> . . .	0	+	
Potassium acid phosphate, <i>M/100</i> .	} Plus sodium car- bonate.	<i>M/10</i> . . . .	0	0	
Magnesium sulphate, <i>M/500</i> . . . . .		<i>M/50</i> . . . .	0	+	
Maltose, <i>M/100</i> . . . . .		<i>M/100</i> . . . .	50	++	
Asparagin, <i>M/500</i> . . . . .		<i>M/200</i> . . . .	90	++	
		<i>M/1,000</i> . . .	<sup>b</sup> 200	+++	
Magnesium sulphate, <i>M/500</i> . . . . .	} Plus potassium acid phos- phate.	<i>M/10</i> . . . .	8	+	
Sodium carbonate, <i>M/100</i> . . . . .		<i>M/50</i> . . . .	0	++	
Maltose, <i>M/100</i> . . . . .		<i>M/100</i> . . . .	50	++	
Asparagin, <i>M/500</i> . . . . .		<i>M/200</i> . . . .	0	±	
		<i>M/1,000</i> . . .	0	0	

<sup>a</sup> 50 in 1.

<sup>b</sup> 295 in 1.

The device adopted is seen to be a very helpful one in determining the value of the various concentrations employed. The cultures in which asparagin was varied show how fortunate a concentration was chosen in the preliminary experiments. Similarly the experience with maltose shows that if asparagin is taken as *M/500* then the maltose must have

<sup>1</sup> I am indebted to my colleague, Mr. J. H. Muncie, for making these readings.

approximately five times the strength. The experiments with magnesium sulphate are contradictory in part, but when the experience on page 742 is considered it may be concluded that for this organism the magnesium-sulphate ratio may be increased with profit. The phosphate proportion represented by  $M/100$  seems to be the favorable one. Sodium carbonate is found to be a constituent entirely unnecessary and for the most part detrimental to fruit-body formation.<sup>1</sup>

By this experiment, which could profitably be carried still farther within the limits indicated, a synthetic culture medium was obtained which gave for this organism a far greater pycnidia production than any other medium tried.

The merits of this medium may now be considered. It is a solution which contains the minerals necessary for growth of a vigorous character, but these chemicals are not present in superfluous amounts. It contains the carbohydrate which gave a remarkably strong, vigorous growth with this fungus, but the amount of the sugar is limited. The nitrogen source is a chemical of known composition and with maltose gave the strongest pycnidium production in the previous experiments. From the behavior of this organism we may conclude that we are approaching an ideal culture medium for the growth and reproduction of this organism. But we may go even farther, since the physiological relations of fungi to the substratum are so much alike. We can safely say that this combination will be found widely useful in producing similar reproduction in related forms. By the application of the same type of manipulation, some such combination can be found for other forms which will give better results than are now obtained on the ordinary media.

We may now consider some of the ordinary laboratory media in their effects upon this organism. The fungus has been cultivated upon a great many of the ordinary materials used in the laboratory for stock cultures and for diagnostic work. In this culture work the relation to light and to oxygen has been carefully observed. The relation to reaction has been but tardily recognized. The experience reported for pea broth shows that almost all relations to media can be reversed by changes in reaction (acidity or alkalinity). The initial relation is not, however, of as much importance as the reaction to phenolphthalein after sufficient growth has taken place to lead to pycnidium formation. Table XXV summarizes the behavior of the organism on the complex media, with the relations on the synthetic solutions included for comparison.

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<sup>1</sup> For convenience the amounts used in preparing this solution may be given. Stock solutions of  $M/5$  chemicals are prepared as follows:

Magnesium sulphate + 7 Aq. 2.466 gm. + 50 c. c. water.

Potassium acid phosphate 1.36 gm. + 50 c. c. water.

Asparagin 1.33 gm. + 50 c. c. water.

Maltose 3.60 gm. + 50 c. c. water.

For 100 c. c. synthetic solution take 1 c. c. of  $M/5$  magnesium sulphate and 5 c. c. of each of the other solutions, and add to 84 c. c. water. Steam on three successive days.

TABLE XXV.—Effect of quality of food: Complex media compared with synthetic solution

Medium.	Reaction.		Growth character.	Aerial form.	Pycnidia.	Time.
	Start.	One month.				
Prune-juice agar (broth from 75 gr.).	+8	±	Strong white mycelium, becoming greenish. Medium reddened.	Strong tufted	+++ <sup>a</sup>	3 weeks.
Glucose agar <sup>b</sup> (glucose 3 per cent; peptone 1 per cent).	.....	.....	White, restricted growth becoming red-brown; oidia.	Prominent.	o	
Corn-meal agar (Shear)	+10	+8	Weak growth of mycelium, mostly submerged; no mat.	Scant, if any	++	4 weeks.
Standard agar.....	+15	—5	Strong growth, white, forming mat.	Scant, if any..	o	
Standard gelatin.....	+20	—5	Strong growth, white, gelatin slowly liquefied.	Fair amount..	o	
Filter paper.....	±	±	Scant amount creeping from point of inoculation, becoming greenish black, paper not discolored.	.....	++	1-3 weeks.
Parsnip plug.....	.....	.....	Strong, quickly covering plug, which shrivels. Color white, then tawny	Tufts.....	+++	4 weeks.
Carrot plug.....	.....	.....	As above, color greenish at close.	Tufts.....	+++	4 weeks.
Pea broth (2 seeds in 10 c. c.).	+8	—8	Strong, forming tough mat, which becomes submerged; white.	Scant.....	o	
Corn broth (2 grains in 10 c. c.).	+8	+5	Scant to medium amount, submerged, forming a film, otherwise no aerial growth, blackening at time of fruiting.	.....	++++	15 - 20 days.
Beans (2 seeds in 10 c. c.)	.....	.....	As in peas.	.....	o	
Bananas (autoclaved).....	.....	.....	Strong, covering slice, reddish brown when old.	Strong.....	+	Slowly formed.
Rice plus 5×water.....	.....	.....	Strong, covering grains, which blacken after a month; white mycelium becoming gray-green.	Strong tufted	o	
Oats (2 grains in 10 c. c.).	+5	±	Weak, submerged growth forming film; blackens in a month.	.....	+	4 weeks.
Raulin solution.....	+30	+20	Strong white, becoming tawny.	Strong tufted	o	
Synthetic solution: Potassium acid phosphate, <i>M</i> /100. Magnesium sulphate, <i>M</i> /500. Maltose, <i>M</i> /100. Asparagin, <i>M</i> /500	+30	+5	[Good white growth, submerged, forming film on surface, on which pycnidia form; mycelium blackens just before fruiting.]	.....	+++++	4-6 weeks.

<sup>a</sup> Aerial<sup>b</sup> One formula calls for 200 gm. of glucose per liter (Harter, 1913).

A comparison of the media with reference to their reaction (+ or —) has already been made. In this relation we have a sharp determining factor which eliminates many preparations. Other media, such as rice, may be taken as cases where a poor balance exists between the nitrogen supply and the carbon supply, thus setting up an unfavorable toxic condition. The corn broth and the synthetic solution behave alike. The aerial growth seems to be strongest in substrata of an acid character. With rich substrata pycnidium production is aerial. The rapid production of pycnidia on filter paper is very significant. The wide range of suitable media is of great importance, and, since these substances must present carbohydrates and nitrogen compounds in great variety,



we have in these complex forms the same sort of result as was obtained in Table XXIII. But, in spite of the variety, the growth is much the same, and when fruiting bodies are produced they are the same morphologically. Such uniformity can be explained only by the assumption of an assimilation process which deals with much the same stuffs in all the substrata. The reserve materials are then worked over by the protoplasm under favorable conditions, and the fructification takes place.

#### EFFECT OF CHANGE OF INTENSITY OF A FACTOR DURING THE GROWING PERIOD

Those experiments of Klebs (1899) in which a bit of rapidly growing mycelium of *Saprolegnia mixta* was transferred from a good nutrient solution to another of poorer quality, with resulting strong response in sporangium production, are the most striking demonstrations of the relation of checked growth to reproductive processes. In experiments of this type we have a device for studying some of the factors with the aim of their further simplification. We must, however, recognize that, no matter how ingeniously the term "checked growth" fits the phenomena described, it really tells us little about the physiological processes underlying.

The following experiment was performed. Strongly growing mycelium (1 week old on corn broth) was washed in two changes of 500 c. c. each of conductivity water. This mycelium was cut in pieces approximately the same size with sterile scissors and was added to the various sterile solutions shown in the table, with the results shown in Table XXVI.

TABLE XXVI.—Effect of change of intensity of a factor: Withdrawal of food supply  
[Time, 1 week]

Medium.	Number of pycnidia.	Growth increment.
1-week-old mycelium added to—		
Filter paper.....	25	++
Conductivity water.....	5	+
Corn broth, 1/40.....	25	++
Corn broth 1X.....	0	++++
Magnesium sulphate, approximately M/100.....	2	+
Pea broth.....	0	+++++
Pea broth, 1/40.....	0	++
Check (similar mycelium allowed to grow undisturbed)...	0	++

It is evident from these results that with the withdrawal of the food supply from vigorous, susceptible mycelium reproduction sets in promptly. The results were obtained in one week—two weeks after inoculation—although normally pycnidium production with corn grains is much slower. This hastening of the reproductive process by change of quantity of food supply indicates that here we were able to produce the change which takes place more slowly in the ordinary cultures.

The following experiment (see Table XXVII), which was performed as part of the experiment given on page 741, gives the effect of change of concentration upon the mycelium. The experiment was made with corn broth and with synthetic solution. The transfer was made after three weeks' growth had taken place.

TABLE XXVII.—Effect of change of intensity of a factor: Change of concentration of food supply

CORN BROTH		
Extent of change.	Number of pycnidia.	Growth increment.
From 10X to $\begin{cases} 10X \\ 5X \\ 1X \\ 1/10X \end{cases}$	2	++
	25	+
	25	+
	25-40	+
From 5X to $\begin{cases} 10X \\ 5X \\ 1X \\ 1/10X \end{cases}$	(a)	.....
	2-25	++
	.....	.....
	.....	.....
From 1X to $\begin{cases} 10X \\ 5X \\ 1X \\ 1/10X \end{cases}$	50	++
	4-9	++
	0	+++
	12-15	+
From 1/10X to $\begin{cases} 10X \\ 5X \\ 1X \\ 1/10X \end{cases}$	.....	.....
	0-3	++
	0	++
	.....	.....
SYNTHETIC SOLUTION		
From 10X to $\begin{cases} 1X \\ 1/2X \\ 1/5X \end{cases}$	0	+++
	100	++
	20	+
From 5X to $\begin{cases} 25X \\ 10X \\ 2X \\ 1X \\ 1/2X \\ 1/5X \\ 1/10X \end{cases}$	0	++++
	0	++++
	0	+++ <sup>b</sup>
	100	+++
	50	++
	50	++
	25	+
From 1X to $\begin{cases} 10X \\ 5X \\ 2X \\ 1/5X \\ 1/10X \end{cases}$	0	+++++
	0	+++++
	0	++++
	15	+
	12	+
	.....	.....
From 1/5X to $\begin{cases} 5X \\ 1X \\ 1/10X \end{cases}$	0	++
	100	+++
	0	+
From 1/10X to $\begin{cases} 2X \\ 1X \\ 1/2X \\ 1/5X \end{cases}$	0	+++
	100	++
	50	++++
	50	+

<sup>a</sup> These transfers were not made. <sup>b</sup> Many immature.

The results given in Table XXVII show in striking manner the effect of the transfer of mycelium from one concentration to another. When mycelium from a poor solution is placed in a rich solution, it begins to grow vigorously, and, on the other hand, when rapidly growing mycelium is transferred to a solution of less concentration, the increase in growth is less. Exactly as the mycelium is checked or started into growth, reproduction is fostered or inhibited. While from the results of the experiments reported before it could only be said that these conditions of growth and reproduction occurred constantly side by side and therefore were related. From this last experiment we have definite proof of the interrelation of these two processes.

Other factors than food supply were experimented upon in the same way. The experiment previously reported under temperature (p. 726) strictly speaking belongs here. It may be remarked that pycnidium production began in the cold before it began in the cultures under room conditions. A similar experiment was performed with corn broth. Corn grains with mycelium about two weeks old, which showed no signs of pycnidium production, were set near a window at room temperature, and in the light in a cold attic where the temperature was about 10° C. After one week there were many pycnidia in the culture in the cold and the growth was checked, while in the culture under room conditions pycnidia production was just beginning and growth had continued regularly. After two weeks, however, the pycnidia were abundant in all the cultures, but were more abundant in the cultures under room conditions. From this experiment it is seen that a checking of growth by other means than food withdrawal can operate in much the same favorable way upon reproduction.

If, then, the factor light, which is known to have a strong power of checking growth, operates in influencing pycnidia production in this manner, we should be able to replace the light effect by checking the mycelial growth in some other way. Cultures, if left in the dark, ought to produce pycnidia eventually. Cultures with scanty food supply, such as those on filter paper, ought to yield pycnidia rather quickly in the dark. The experiments already reported have failed to show this action. Therefore, the action of light is not merely due to the checking influence which it has upon mycelial growth. If it were, we should have the paradoxical condition in which the withdrawal of light from a culture with limited food supply would augment pycnidium production, because of the greater growth in the dark and the more rapid diminution of the nourishment.

The following experiment (see Table XXVIII) was performed, in which the effect of checking the growth of corn-broth cultures by low temperatures was tried in both light and dark conditions. Corn-broth cultures 12 days old were placed under the conditions shown in the

table. The cultures in the dark were placed in the dark chambers described on page 723, and those in the light were placed in battery jars with tilted covers.

TABLE XXVIII.—*Effect of change of intensity of a factor: Change in temperature*

Conditions	Pycnidia.		Growth increment, two weeks.
	One week.	Two weeks.	
Room temperature (22°):			
Dark.....	o	o	++++
Light.....	o-4	25-50	++
Approximately 10°:			
Dark.....	o	o	++
Light.....	10-15	10-25	++

The conditions were continued for two weeks longer without any change in the relations. This experiment reinforces the conclusion just arrived at that light has some other action than a mere checking of growth, and its action can not be replaced by a mere checking of growth.

Light is known to have a powerful oxidizing effect, and organic material under the influence of light is subjected, according to Freer and Novy (1903), to the action of organic peroxids engendered by the catalytic action.

The following experiment was tried to determine whether some such action was concerned. Hydrogen peroxid was added to 12-day-old corn-broth cultures at the rate of 2 drops (1/20 c. c.) of a 3 per cent solution to a dish. The experiment was checked with cultures of the same age. The dishes were placed in a dark chamber. After a week (first examination) the result shown in Table XXIX was obtained.

TABLE XXIX.—*Effect of change of intensity of a factor: Addition of hydrogen peroxid to corn broth*

Medium.	Pycnidia.
Corn broth + hydrogen peroxid (H <sub>2</sub> O <sub>2</sub> ).....	+ a
Corn broth, check.....	—

a<sub>4</sub> to 8.

By strongly oxidizing cultures with hydrogen peroxid it was possible to replace the morphogenic action of light. Light, therefore, must act in some such manner upon this organism, and the action in fruit-body formation must be of some such character. This experiment was repeated at least six times, with varying concentrations of hydrogen peroxid. With cultures grown in the dark for from two to three weeks, the addition

of from 1/25 to 1/5 c. c. of hydrogen peroxid (3 per cent) would produce a few pycnidia with darkened cultures. In the stronger concentrations the mycelium was completely enveloped with a froth. After the first stimulation the cultures produced no further pycnidia. It must be said that in no case were pycnidia produced in amounts equal to those under light conditions. At best the use of hydrogen peroxid is a very harsh method.

With young cultures or with very old cultures the hydrogen peroxid was ineffective. In these its behavior is like that of light.

Other chemicals known to be strong oxidizing agents were employed. It may be said that nearly all gave positive results at extremely weak dilutions, provided that the mycelium used was in proper condition. Mycelium which would produce pycnidia by an hour's exposure to light gave good results with the oxidizing agents.

Another factor was doubtless responsible for the inequality of pycnidium formation in these experiments. All the chemicals used are toxic to the mycelium. In the concentrations used, these poisoned the cultures and certainly affected the reactions.

Table XXX summarizes the successful trials.

TABLE XXX.—*Effect of change of intensity of a factor: Use of various chemicals*

Chemical and concentration.	Corn broth.	Synthetic.	Pea.
Nitric acid ( $\text{HNO}_3$ ), $M/500$ .....	+	+	+
Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), $M/500$ .....	+	+	—
Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), $M/500$ , + potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ), $M/500$ .....	+	+	—
Potassium permanganate ( $\text{K}_2\text{Mn}_2\text{O}_7$ ), $M/500$ .....	—	—	—
Ferric chlorid ( $\text{FeCl}_3$ ), 1 drop of $M/5$ .....	+	+	—
Zinc sulphate ( $\text{ZnSO}_4$ ), $M/500$ .....	—	—	—

#### GENERAL DISCUSSION

The work reported in this paper has given more or less of a definition of the environment in which *Plenodomus fuscomaculans* can live and reproduce. We now know the bare essentials for growth—the base level of existence—since we know the minima of the various formal conditions of growth. Similarly, we know some of the highest intensities which can be tolerated.

For growth at the base level of existence, there is only required the almost immeasurably small food supply of conductivity water, a scanty amount of free oxygen, and a temperature of 6° C.—perhaps lower. These factors may be increased in intensity until there is tolerated a food supply enormously larger, abundant oxygen, and temperatures up to 37° C.—perhaps higher. But as the simple minimum conditions are passed, the interactions of the component factors of the environment increase, and new factors arise which also have their limits. With

increase of food supply we must now consider, besides the mere chemical parts, the ratio of these parts to each other, both at the outset of growth and throughout the growing period. We analyze such relations and classify them as reaction, etc.

For pycnidium production the limits are found to be much narrower than those suitable for growth. No reproduction takes place at the base level of existence. Food supply must be increased, not greatly, but in measurable amount. From the scanty supply in conductivity water it must increase to the quantity found in distilled water—a two-fold to tenfold increase. Or it must be present in at least one thousandth of the quantity cooked from a few sheets of finest filter paper by conductivity water, but one-tenth of this amount is not sufficient. Oxygen must be present in abundance; stagnant air prevents reproduction. The temperature may be as low as  $10^{\circ}\text{C}$ ., but must not be as low as  $6\frac{1}{2}^{\circ}\text{C}$ .

Up to a certain limit (perhaps up to  $M/50$ ), increase in concentration of the food supply augments reproduction. After that point the excess food supply retards and eventually inhibits reproduction. Fructification, when it does take place with media of higher concentration, takes place in the aerial mycelium, and doubtless here the conditions are comparable to those in which the fructification is produced within or upon the medium.

The kind of food may vary almost without limit. An organism which can grow and reproduce in distilled water or a grain of corn can find requisite food materials in almost any biological product. But the more complex substances bring new relations, which, while of some importance to growth, are of decisive importance for reproduction. Growth can take place between the acid and alkali limits of  $+30$  and  $-10$  to phenolphthalein, but reproduction is limited to the conditions but slightly more acid than the neutral point of this indicator.

Corn broth seems at first glance a better foodstuff for this organism than oat broth, and in two parallel cultures the first will produce 50 pycnidia while the other is producing one. Yet if the oat culture be acidified with an acid phosphate, or even with hydrochloric acid, it becomes nearly as good a culture medium as the corn. Glucose agar made after the ordinary formula gives a strong growth with this organism, but no pycnidia. If the chemicals of this formula be diluted 50 times, the organism will fruit abundantly upon it. This organism was found to be greatly overfed by the ordinary laboratory media, and under the influence of the great excess of food grew and grew until the by-products of metabolism checked growth or destroyed the organism.

The differences in media were not so much in the food which they contained—for an examination of published analyses will show all necessary elements for growth and reproduction in almost any plant—as in the acid or alkaline reaction which the medium gave when prepared, the reaction maintained, and the concentration or relative scantiness of carbohydrate

and protein. The least adapted synthetic solution for this fungus (Raulin, 1869), could be made to yield pycnidia by the addition of lime, which probably counteracted the acidity; and pea cultures in which the mycelium was submerged and nearly dead could be made to grow and produce pycnidia by mere acidification. Furthermore, pea cultures to which sugar is added to balance the protein produce abundant pycnidia in the aerial hyphæ.

A consideration of the various laboratory media shows them to be rather purposeless, clumsy devices, in which this organism is overfed. Except the very simplest ones, none have warrant for existence if considered from the point of view of adaptability for a specific purpose. The great similarity of results on the various media seems to require the conclusion that these foodstuffs are not specific. Any fruit or vegetable is a full nutrient for almost any organism if the material be made properly soluble, and any harmful acid or alkaline reaction or otherwise unfavorable concentration be adjusted. Probably any biological product can likewise be utilized. Our methods have made a fetish of variety and have completely neglected the contributing factors.

As has been said, fungi behave alike in their relations to the substrata in the vast majority of cases. That the findings for this organism apply to others seems entirely probable. In many ways confirmatory evidence is to be found in the present practices. A certain medium is discovered which gives fruiting bodies for some fungus. A number of other organisms not at all related, in spite of differences in life relation, are also found to fruit upon this medium.

A consideration of one of the best preparations devised for fruit-body formation is very interesting. Shear's corn-meal agar is made by stiffening with agar the infusion obtained from four teaspoonfuls of corn meal (Shear and Wood, 1913). This medium is suitable for fructification for this organism, because it gives a scanty food supply, yet sufficient readily available to produce the growth necessary for pycnidium formation. The ratio of carbohydrate to protein is such that the reaction remains acid. Reasoning from such similar phenomena, a rather general application may be made. Any organism of this type can be made to grow and fruit upon a synthetic substratum containing the essential components, provided that the ratio of the components, hence the acid or alkaline reaction, and the concentration, be adjusted to the limits demanded by the particular organism. This assumes that the factors of light, temperature, aeration, etc., also fall within their own suitable limits.

We have, therefore, within the reach of experimental work the possibility of developing an environment which can be so defined that it can always be duplicated, suitable for a great group of organisms (Thom, 1910). With such a chemically and physically defined environment the classification of organisms could be placed upon a sounder working basis.

It is commonly admitted that the description of an organism must be taken under the assumption of some definite environment. The great mass of media in common use, the uncertainty of composition, the lack of standardization, and the usual failure to bring about fructification have left the description of fungi with only the natural habitat as a fixed environment. With forms of comparatively simple morphology this standard has led to the classification by hosts, with its attendant multiplicity of species. A firm basis for taxonomy can be arrived at, and simplification can come, only from a standardized environment.

As has been indicated in the preceding discussion, the physical environment must also be defined. With the growth of our knowledge of the forms we shall be able to a great extent to analyze the complex of forces. In the present paper one such force has been emphasized and its action discovered to be related to the liberation of energy by oxidation.

Light was found to be essential for reproduction. If light be absent or insufficient, although all other requirements were satisfied—with a medium suitable for growth and food supply, aeration, acid reaction, temperature, all within the proper limits—pycnidium production will not take place. Instead, aerial mycelium is formed, and eventually the organism goes into a static condition. The light factor, as others, has its limits. Weak light will not allow pycnidium production. This factor differs from the others in that its action need not be continuous. It is therefore of direct stimulative nature. A short exposure to strong diffuse light of cultures from dark conditions, which are otherwise ready for pycnidia formation, gives the necessary stimulus during a further period in the dark. When the effect of the stimulation is spent in the production of a few pycnidia, a second exposure is necessary for a second inauguration of the process.

The action of light in thus unlocking these forces is very satisfactorily explained by the experiment in which a few drops of hydrogen peroxid were used to replace the light stimulus. Other oxidizing agents also serve to stimulate fruit-body formation. The protoplasm of well-nourished mycelium is rich in oily reserve materials, and the action of light may oxidize these bodies and change them from emulsions of poor mobility to materials of great diffusibility. Accompanying this we have a releasing of energy, and fruit-body formation is inaugurated. The mechanism of this process is not known at all, but Herzog (1903) has shown that the sporulation of yeast is affected by temperature, and the curve for the variation in amount produced by temperature is a typical enzyme curve.

Hydrogen peroxid added to a pea-broth culture, to a rich sugar solution, or to a young growing culture on corn broth does not immediately lead to fruit-body formation, nor does the action of light on such cultures lead to it. The action of light is modified and controlled by the condi-



tion of the mycelium, and this we have seen is a resultant of the environmental factors. In other words, we must consider in what way a mass of mycelium with checked vegetative growth is influenced to reproduction, while one in active growth is unaffected.

The cause of this relation to light, or, better, to oxidation, is understood if we take into account the fact that among organisms and among parts of the same organism there exists a strong competition for oxygen. In the cell itself the various processes inhibit and influence each other by their oxygen relations. Oxidation is never at its maximum in the cell under ordinary conditions, as simple tests with increased oxygen tensions show (Porodko, 1904). Organisms well aerated grow better than those in an air supply below the optimum. The action of oxidation is to release energy. The materials oxidized are either the foodstuffs suitable for nutrition or the cell material which growth has stored up. Euler (1909) contrasts growth, a stretching process, with reproduction, a differentiating and formative process. Growth is a process which is gradual, and it takes place even if only a small amount of energy be available. It is a process taking less energy than reproduction, as all respiration experiments have shown. The great consumption of energy in reproduction is doubtless associated with the great amount of nuclear protoplasm which must be formed. Growth, therefore, is the process first inaugurated, and the one which continues so long as the food supply is abundant and outer conditions permit. It is a static condition, as reproduction is dynamic.

In the hunger state the oxidations are different to a marked degree, as Kosinski (1901) has discovered, and here we have the cell reserve gradually drawn upon. The fats and even the proteins may be oxidized, according to Purievich (1900). But in this hunger state the respiration is reduced, according to Kosinski; hence, the working is slow. These metabolic relations, in spite of their great complexity, balance each other.

It would seem that reproduction is not possible under conditions favoring growth, because the oxygen supply is all used in ordinary metabolism. With the hunger state, respiration is reduced. Oxidation becomes vigorous if it be stimulated by light. No doubt any catalytic agent would be similarly effective. Once in this hunger state, oxidation, if augmented, takes place upon the rich cell stuffs, with the liberation of much energy. This energy is used in reshaping the reserve stuffs into complex protein bodies—the spores. The sharper the hunger condition is made, the more striking the reaction in pycnidium production. The sudden withdrawal of the food supply by the transfer of richly-growing mycelium to lower concentrations or to distilled water, checks ordinary assimilation, with its attendant use of oxygen. If oxidation of the cell reserves be inaugurated by light or some strong oxidizing agent, fructification takes place.

We may now consider other factors in the light of this theory. Experiment has shown that aeration is essential for reproduction. The action of light upon the protoplasm is dependent upon the oxygen supply. Aeration may work to continue the oxidizing process by the removal of end products, thus allowing oxidations to proceed to completion. In many cases recorded in the literature the effect of transpiration is to further the exchange of gases. The action of low temperature was to check growth, and pycnidium production was found to start. Euler (1909) states that lowering the temperature affects the oxidation process to a lesser degree than it affects other processes.

The action of the acid reaction is interesting and confirmatory. So far in this discussion the mechanics of the oxidation have not been considered. Oxidations in plants are generally believed to take place through the activity of oxidases of various sorts. As is well known, light activates this type of enzyme, although it is detrimental to such enzymes as diastase (Euler, 1909, p. 97). The pronounced and sudden blackening of cultures about to produce pycnidia is very significant and can be best explained by the oxidation of some leuco compound by an oxidase (Kruse, 1910, p. 787). Some oxidases are known which work better in a slightly acid medium. We have seen that for this organism an alkaline medium was prejudicial to reproduction. The effect of acid reaction in favoring the reproductive process has not been explained, but it may have some connection with the enzymotic process. At any rate, an oxidation of oily stuffs to fatty acids would give a medium suitable for further activity of these ferments.

The formation of pycnidia in the aerial mycelium and in fact the whole series of complex reactions which Klebs (1900) has associated with "*Luft leben*" become much more comprehensible if we view them from the point of view of oxidation.

The replacement of the light factor by hydrogen peroxid thus becomes of great importance in reducing to simple terms the phenomena encountered. Light can unlock in suitable mycelium the reproductive process. This it does by its catalytic influence. The action may be due to the activation of oxidases along with the inauguration of a reaction (acid) favorable to their continued action; but this oxidation thus set up does not proceed to reproduction if the growth process is consuming the energy. If growth is not able to proceed, owing to scanty food supply or some checking influence, then the catalytic action of light inaugurates a building of the stored foodstuffs into complex fruiting bodies.

This general discussion may now be summarized. In the historical portion of the paper it was seen that the environment may be viewed as a directive and collective force which can be utilized for unfolding the life history of an organism. The great generalizations of Klebs are broad, and by their very broadness make possible acceptance in a wide

range of cases. Their teachings can not, however, be made the basis for research without the development of methods of attack suitable to a series of forms. The method of this paper may be used for similar organisms.

The first part of the paper may be interpreted as a determination of the limits of the life processes, which, when once determined, allow in the latter part of the paper a manipulation of them. The knowledge of the factors and their optima made possible a development of an environment especially fitted for growth and reproduction.

The proposition of Klebs, that the limits of reproduction are narrower than those of growth, is fully substantiated. Klebs further pointed out that growth and reproduction are processes opposed to each other. This is true for the organism studied.

The action of light has led to an insight into the mechanism of this opposed action. It has shown that growth, the static condition, is opposed to reproduction, a dynamic condition. Where one process is storing energy, the other is a process consuming energy. The equilibrium within the cells needs to be upset by some oxidizing force in the case of this fungus to inaugurate fruit-body formation in susceptible mycelium.

It is not concluded from the experiments with this species that light is a specific factor which will cause reproduction to take place in all forms, once growth is checked, although it may be expected to be an important condition in related organisms. But, in view of the great similarity of behavior in all the forms tested so far with respect to growth and reproduction, it may be concluded that in them some stimulus becomes operative when an organism is in the hunger state which starts the utilization by oxidation of the stored food supply and leads to the phenomenon of reproduction.

#### SUMMARY

This paper gives the results of experiments performed with *Plenodomus fuscomaculans*, a fungus pathogenic to the apple. The specific problem undertaken was the determination of the effects of various controlled environmental factors upon the growth and reproduction of this fungus.

The historical development of the art of culturing organisms has been traced from the first crude cultures to the present elaborate technic. The simultaneous development of our knowledge of the physiology of organisms has been briefly summarized. This survey shows that the environmental factors may greatly influence the life processes of organisms. Organisms have been cultured in the laboratory in an imitative or haphazard way, with a chance of finding a suitable environment. Owing to the great variety of available methods and the great plasticity of organisms, this course has been productive of results with some forms. Another type of research has sought to find the relation of the organism

to its environment and by manipulation of the environmental factors to discover the various phases of life history. Although many related forms have been grown in pure culture, very little physiological work of this type has been done with the Sphaeropsidales.

The organism was found to have a wider range of conditions suitable for growth than for reproduction. The base level of conditions necessary for growth is found in conductivity water at low temperatures. Reproduction requires more favorable conditions. Pycnidium production took place only in cultures exposed to light. The ordinary room temperatures were sufficient. Abundant aeration is essential. Transpiration is a factor of secondary importance. A slight acid reaction, especially at the close of the growing period, is a necessary condition. The value of a medium depends largely upon the acid or alkaline reactions present, not alone at the beginning but at the close of the growing period. Autointoxication was observed and was traced to excess of either acid or ammonia, which was the product of too great a proportion of either carbohydrate or protein, respectively.

As has been said, the quantity of foodstuff necessary for growth is extremely minute. Pycnidium production requires more food, but the meager amount present in distilled water is sufficient to allow the production of a few pycnidia. On the other hand, the fungus is able to tolerate very rich food supplies, but pycnidium production in solutions is restricted to  $M/100$  or perhaps  $M/50$  sugar concentration. Exact limits are hard to determine, because of the formation of mats or films in solutions, which effectively wall off much of the food supply. Fructification in the case of rich media takes place in the aerial hyphæ, and no doubt this relation corresponds with the conditions in solutions.

Magnesium sulphate and potassium dihydrogen phosphate in very dilute solutions furnish the necessary mineral elements for growth and reproduction. The carbon supply may be taken from a wide range of compounds of alcoholic structure. The carbohydrates furnish food materials in most available form, and, of these, xylose and maltose produce the best growth. The carbohydrates do not seem to be specific in producing fruiting bodies, and almost any are suitable if taken at the right dilution. The nitrogen assimilation is greatly influenced by the type of carbon nutrition.

The minerals mentioned and maltose and asparagin at the ratio of 5 to 1 seem to offer the most favorable combination, although others are suitable. From the experiments a medium was selected which though of entirely known composition gave better growth than any other tried. This synthetic solution had a scant amount of food supply, yet enough to permit a quick, vigorous growth. It retains the acid reaction till the close of the growing period. A study of this medium gave a basis for a criticism of results obtained with the common laboratory combinations.

The problem of this paper was a study of the effect of environmental factors upon this organism, especially as they influenced growth and reproduction. The experiments here reported verify the conclusions of Klebs and extend them for an untested group of organisms, the Sphaeropsidales. As has been pointed out, in this paper the method of approach was different from the inductive methods used by Klebs in drawing his conclusions, since the methods employed here were deductive, based on our knowledge of the reactions of other organisms. The experiments with *Plenodomus fuscomaculans* give a method applicable to related forms. The results of this physiological work give a basis for practical recommendations as to the culture of other organisms, as well as evidence of the feasibility of developing a standard synthetic solution which would make possible a standardization of environments for diagnostic purposes.

The action of light, when pushed to a last analysis and when considered in view of the experiment in which hydrogen peroxid and other oxidizing agents replaced it, is seen to be of either an oxidizing or a catalytic type. This led to the development of a theory to explain the mechanism of the opposed action of growth and reproduction. This theory sees in the competition for oxygen the fundamental reason for the absence of fructification under conditions which allow abundant growth.

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# EFFECT OF ELEMENTAL SULPHUR AND OF CALCIUM SULPHATE ON CERTAIN OF THE HIGHER AND LOWER FORMS OF PLANT LIFE<sup>1</sup>

By WALTER FITZ,  
*Assistant Agricultural Chemist, Agricultural Experiment Station  
of the University of Wisconsin*

## INTRODUCTION

A study of the literature<sup>2</sup> shows that a number of investigators have noted a beneficial effect when elemental sulphur or sulphates are added to certain soils. The number of these investigations and also the types of soil and plants employed are limited. Certain workers report no beneficial effects from the addition of sulphur or sulphates to soil, and in isolated cases an injurious effect has been noted. Just how the sulphur or its compounds act is little understood, but there are two plausible explanations: (1) That it acts as a fertilizer, supplying the sulphur needed for plant growth, and (2) that it acts as a corrective agent—i. e., it favors beneficial groups of bacteria, while injurious forms are retarded in growth. However, the problem of sulphur and sulphates in agriculture is still far from being solved. This is especially true in the case of the effect of sulphur and sulphur compounds upon micro-organisms. In order to study this phase of the problem, a series of experiments was planned.

## PLAN OF WORK

The object of these experiments was (1) to note the effect of sulphur and sulphates upon the soil micro-organisms and on pure cultures of legume bacteria, and (2) to note the effect of sulphur and sulphates upon the growth of red clover (*Trifolium pratense*).

For the experiments with mixed cultures, fresh soil was used as an inoculum. For legume bacteria all materials were sterilized, and the nutrient medium was inoculated with a pure culture of bacteria from the nodules of red clover.

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<sup>1</sup> Paper from the Laboratories of Agricultural Bacteriology and Agricultural Chemistry of the University of Wisconsin.

<sup>2</sup> Hart, E. B., and Tottingham, W. E. The relation of sulphur compounds to plant nutrition. *In* Jour. Agr. Research, v. 5, no. 6, pp. 233-250. 1915. Literature cited, p. 249.

# EFFECT OF ELEMENTAL SULPHUR AND SULPHATES ON SOIL BACTERIA

## MIXED CULTURES

For these experiments ten 1-gallon jars containing 2 kgm. each of Miami silt loam taken from the Wisconsin Experiment Station farm were used. The analysis of this soil is as follows:

	Per cent.
Potassium.....	2. 16
Nitrogen.....	. 15
Phosphorus.....	. 15
Sulphur.....	. 016
Calcium carbonate.....	. 33
Humus.....	1. 38

The moisture content of the soil was held at 18 per cent, or about half-saturation. Each jar was covered with a layer of cotton and gauze to prevent contamination, and was incubated at 28° C. Various amounts of sulphur and of calcium sulphate were added to the pots, as shown in Table I. At definite intervals samples were taken from the jars and bacterial counts as well as determinations of ammonia and of nitrates made. The results of the latter are given in Table I.

TABLE I.—*Effect of calcium sulphate and elemental sulphur on soil bacteria*

Treatment.	Number of organisms per gram of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated.....	6,866,000	8,746,000	10,790,000	6,350,000	10,782,000
Given 0.01 per cent of calcium sulphate ..	6,866,000	10,544,000	13,900,000	6,590,000	11,026,000
.05 per cent of calcium sulphate ..	8,506,000	14,140,000	13,789,000	8,028,000	9,945,000
.10 per cent of calcium sulphate ..	7,221,000	7,923,000	13,060,000	7,923,000	9,824,000
.50 per cent of calcium sulphate ..	8,290,000	8,029,000	13,420,000	7,548,000	10,305,000
1.00 per cent of calcium sulphate ..	8,580,000	9,585,000	12,938,000	7,068,000	9,945,000
Untreated.....	6,590,000	9,166,000	8,626,000	6,949,000	9,705,000
Given 0.01 per cent of sulphur ..	7,429,000	8,746,000	8,866,000	7,923,000	8,626,000
.05 per cent of sulphur ..	9,106,000	8,866,000	10,065,000	7,668,000	8,116,000
.10 per cent of sulphur ..	8,290,000	8,208,000	10,300,000	6,590,000	8,866,000
.50 per cent of sulphur ..	8,864,000	11,020,000	4,914,000	3,194,000	2,995,000
1.00 per cent of sulphur ..	6,504,000	7,070,000	2,635,000	2,329,000	719,000

The data show that calcium sulphate in the quantities used apparently has little effect on the number of soil organisms. Elemental sulphur, however, decreases the number of soil organisms that grow on agar plates. This decrease is not noticed until after 44 days, and only in soils to which 0.05 and 1 per cent of sulphur had been added. Quantitative acidity tests of the soils of these two jars showed it to be distinctly acid. This is corroborated by the work of Lint,<sup>1</sup> who has shown that in soil elemental sulphur is oxidized to sulphate and that the acidity produced is proportional to the amount of sulphur added. Acidity determinations were made according to Truog's<sup>2</sup> method and are given in Table II.

<sup>1</sup> Lint, H. C. The influence of sulphur on soil acidity. *In Jour. Indus. and Engin. Chem.*, v. 6, no. 9, p. 747-748. 1914.

<sup>2</sup> Truog, E. A new test for soil acidity. *Wis. Agr. Exp. Sta. Bul.* 249, 16 p., 3 fig., 1 pl. 1915.

TABLE II.—*Acidity of soil treated with elemental sulphur*

Treatment.	Calcium oxid necessary to neutralize acid in 10 gm. of soil.
	Gm.
Untreated.....	0.0000
Given 0.01 per cent of sulphur	.0000
.05 per cent of sulphur	.0000
.10 per cent of sulphur	.0011
.50 per cent of sulphur.	.0369
1.00 per cent of sulphur.	.0668

The results of these determinations show that the acidity produced by the oxidation of elemental sulphur to sulphate is proportional to the amount of sulphur added. In the samples to which 0.01 and 0.05 per cent of sulphur had been added, the soil contained enough lime to neutralize the acidity.

Change in reaction is probably the cause of the decrease in the number of the soil organisms. Abundant mold growth was found on the surface of the acid soils.

Table III shows that calcium sulphate in the quantities used has no effect on the production of ammonia in the soil. Elemental sulphur, however, in concentrations of 0.5 and 1 per cent increases the production of ammonia to a marked degree. This increase is noticeable after 44 days.

TABLE III.—*Effect of calcium sulphate and elemental sulphur on the production of ammonia in the soil*

Treatment.	Quantity (in milligrams) of ammonia nitrogen per 100 gm. of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated.....	3.99	3.19	3.40	3.21	3.23
Given 0.01 per cent of calcium sulphate....	3.19	2.38	3.32	2.80	3.48
.05 per cent of calcium sulphate....	3.19	2.21	3.06	3.06	3.57
.10 per cent of calcium sulphate....	3.82	2.38	3.23	3.32	3.23
.50 per cent of calcium sulphate....	3.19	2.29	3.36	3.40	3.57
1.00 per cent of calcium sulphate....	3.82	2.21	3.06	3.73	3.40
Untreated.....	3.97	3.19	3.12	2.97	3.40
Given 0.01 per cent of sulphur....	3.91	2.38	3.19	2.72	3.23
.05 per cent of sulphur.....	3.19	2.29	3.06	2.46	3.23
.10 per cent of sulphur.....	3.06	2.21	3.23	2.55	3.16
.50 per cent of sulphur.....	3.82	2.89	5.95	7.11	8.26
1.00 per cent of sulphur.....	3.95	2.89	6.80	7.31	9.52

The data in Table IV show that calcium sulphate in the quantities used does not materially affect the formation of nitrates in the soil. Elemental sulphur, on the other hand, in concentrations of 0.5 and 1 per cent decreases nitrate formation. This decrease is noticeable after 30 days. Previous to this time the sulphur does not seem to injure nitrate formation. Concentrations of sulphur lower than 0.5 per cent have no

appreciable effect on nitrification. It should be noted that while the bacterial counts begin to decrease after 44 days, the ammonia content begins to increase at this time.

TABLE IV.—*Effect of calcium sulphate and elemental sulphur on nitrate production in the soil*

Treatment.	Quantity (in milligrams) of ammonia nitrogen per 100 gm. of soil after—				
	12 days.	30 days.	44 days.	72 days.	93 days.
Untreated.....	1.87	1.34	2.95	3.93	4.70
Given 0.01 per cent of calcium sulphate ..	2.12	1.25	2.35	4.13	5.07
.05 per cent of calcium sulphate ..	2.42	1.01	2.49	4.58	5.72
.10 per cent of calcium sulphate ..	1.14	1.25	2.10	4.03	5.47
.50 per cent of calcium sulphate ..	1.86	1.23	2.45	2.94	4.99
1.00 per cent of calcium sulphate ..	1.35	1.82	2.39	2.87	4.51
Untreated.....	1.53	1.66	2.93	3.93	4.35
Given 0.01 per cent of sulphur.....	1.80	1.99	2.65	3.13	4.13
.05 per cent of sulphur.....	2.17	1.88	2.29	3.20	4.53
.10 per cent of sulphur.....	1.38	1.69	2.92	4.13	4.93
.50 per cent of sulphur.....	1.24	.54	1.41	1.14	.89
1.00 per cent of sulphur.....	.94	.54	.64	.95	.85

#### PURE CULTURES

In order to determine the effect of calcium sulphate on pure cultures of legume bacteria (red clover), Ashby's solution, minus the sulphate, was used. To 100 c. c. portions of this solution in 10 large Erlenmeyer flasks were added 30 gm. of pure quartz sand and various amounts of calcium sulphate. The sand was used to aid in breaking up the aggregates of bacteria when samples were taken for counts. All cultures were incubated at 20° C., and at intervals of one and two weeks bacterial counts were made. The results of these counts are given in Table V.

TABLE V.—*Effect of calcium sulphate on the growth of red clover organisms in Ashby's solution*

Treatment.	Number of organisms per cubic centimeter of solution after—		
	0 day.	7 days.	14 days.
Untreated.....	30,000	53,000,000	157,000,000
Given 0.01 per cent of calcium sulphate.....	30,000	139,000,000	425,000,000
.02 per cent of calcium sulphate.....	30,000	177,000,000	400,000,000
.05 per cent of calcium sulphate.....	30,000	198,000,000	450,000,000
.10 per cent of calcium sulphate.....	30,000	121,000,000	350,000,000

The data show that the numbers of bacteria that grow on Ashby's agar were increased by the addition of calcium sulphate. The increase is very marked after both 7 and 14 days. It should be noted that 0.01 per cent of calcium sulphate is apparently just as efficient in producing an increase in the number of bacteria as is 0.1 per cent. This seems to indicate that only a trace of calcium sulphate is needed to stimulate the legume bacteria.

This experiment was repeated, using soil solution in place of Ashby's solution. For this purpose 1 kgm. of Miami silt loam was placed in a large container, 1 liter of distilled water added, and the entire mass boiled for one hour. It was next filtered, and 0.05 gm. of dipotassium phosphate and 1 gm. of mannite were added. This was then put into ten 500 c. c. flasks and 30 gm. of quartz sand added. Various amounts of calcium sulphate were used. The flasks were sterilized, and when cool were inoculated with a pure culture of red-clover bacteria. All cultures were incubated at 23° C. At intervals of one, two, and three weeks bacterial counts were made. These results are given in Table VI.

TABLE VI.—*Effect of calcium sulphate on the growth of red-clover organisms in soil solution*

Treatment.	Number of organisms per cubic centimeter of solution after—			
	0 day.	7 days.	14 days.	36 days.
Untreated . . . . .	180,000	63,000,000	145,000,000	146,000,000
Given 0.01 per cent of calcium sulphate . . . . .	180,000	135,000,000	176,000,000	217,000,000
.02 per cent of calcium sulphate . . . . .	180,000	125,000,000	178,000,000	244,000,000
.05 per cent of calcium sulphate . . . . .	180,000	135,000,000	269,000,000	249,000,000
.10 per cent of calcium sulphate . . . . .	180,000	138,000,000	185,500,000	262,000,000

From the data it is evident that the addition of calcium sulphate stimulates the growth of red-clover organisms in pure cultures to the extent of more than 100 per cent. The results of this test agree with those obtained in Ashby's solution—i. e., that small amounts of calcium sulphate are apparently as beneficial as larger amounts.

#### EFFECT OF SULPHUR AND SULPHATES ON HIGHER PLANTS IN ARTIFICIAL MEDIA

Various experiments were made with the view of determining the effect of calcium sulphate and sulphur upon the growth of clover and upon nodule formation. This was tested first in artificial media. The medium consisted of a soft synthetic agar prepared from 1 liter of tap water, 5 gm. of dipotassium phosphate, and 7 gm. of agar. This medium was sufficiently firm to support the seeds. Thirty c. c. of the melted agar plus various quantities of calcium sulphate were added to each of 50 test tubes. In order to reduce the individual variation between the plants, 10 parallel tubes were used. The tubes were sterilized, and then two seeds of red clover were planted in each. After inoculation the cultures were removed to the greenhouse. At the end of two weeks greater root development was noted in the calcium-sulphate test tubes than in the untreated ones. In the older plants the increase in root development became most marked. The tops, however, failed to show any difference in size. In the tubes to which 0.1 per cent of calcium sulphate had been added, the plants were slightly smaller than the

others. At the end of six weeks the plants were removed and the roots measured. There was a distinct difference in root development, as shown in Table VII. Plate LVI, figure 1, shows very plainly the decided differences in root development. The results indicate that the increase in root development is as great with only 0.01 per cent of calcium sulphate added as with larger amounts. The test tubes treated with calcium sulphate were chosen at random from the calcium-sulphate series. They appear lighter because of the suspension of small particles of the salt in the agar.

The results of this experiment show that calcium sulphate greatly increases root development. However, in concentrations as high as 0.1 per cent, growth is slightly retarded. The increase in root development may be of considerable importance, first, because it enables the plant to reach out over a greater area for nourishment, and second, because of the greater field, the plant will be able to withstand drought better and thrive on poorer soil. The increase in root development may be the cause of the increase in the yield of clover when calcium sulphate is added to the soil. This is in confirmation of the work of Hart and Tottingham.<sup>1</sup>

These results are given in Table VII, which represents the average of 10 test tubes for each concentration used.

TABLE VII.—*Effect of calcium sulphate on the growth of red clover*

Treatment.	Length of root.	Length of stem.
	Cm.	Cm.
Untreated .....	3.8	4.2
Given 0.01 per cent of calcium sulphate .....	5.1	4.19
.02 per cent of calcium sulphate .....	5.5	4.7
.05 per cent of calcium sulphate .....	5.01	4.6
.10 per cent of calcium sulphate .....	4.93	3.3

#### EFFECT OF SULPHUR AND CALCIUM SULPHATE UPON CLOVER GROWN IN VARIOUS TYPES OF SOILS

The effect of calcium sulphate upon clover grown on Miami silt-loam soil was tested. For this experiment ten 1-gallon jars were used. Four kgm. of Miami silt-loam soil and various amounts of calcium sulphate were added to each. The jars were kept in the greenhouse and the moisture content held at 18 per cent. Each jar was seeded with red clover and then inoculated with a pure culture of red-clover organisms. After two weeks the jars were thinned to 10 plants.

During the first few weeks there was no apparent difference in the size of the plants. At the end of seven weeks an increase in growth in jars 3 to 8, inclusive, was noted. In jars 9 and 10, to which 0.1 per cent of calcium sulphate had been added, there was a decrease in growth. Four

<sup>1</sup> Hart, E. B., and Tottingham, W. E. Op. cit.

representative plants were removed from each jar. The roots of the plants grown in the sulphate-treated soil were longer and more branched than those of the plants grown in the untreated soil. There was an apparent increase in the number of nodules grown in the sulphate-treated series, except in the case of plants grown on soil to which 0.1 per cent of calcium sulphate had been added. The number of nodules on the above plants were about the same as on the plants grown in untreated soil. It must be remembered that the plants grown in the soil containing 0.1 per cent of calcium sulphate were smaller and therefore would naturally contain fewer nodules than the larger plants. Plate LVI, figure 2, illustrates these effects. The plants in group A were taken from the untreated soil; B, from the soil to which 0.01 per cent of calcium sulphate had been added; C, from soil to which 0.02 per cent had been added; D, from soil to which 0.05 per cent had been added; and E, from soil to which 0.1 per cent of calcium sulphate had been added. Note the marked increase in root development in B, C, D, and even E, where the plants are the same size as those in group A; also note that group E, to which 0.1 per cent of calcium sulphate was added, and D, to which 0.05 per cent was added, show no greater growth than A, the untreated, while groups B and C show an increase in top as well as root. The illustration shows very distinctly the increase in length of root and also the decrease in the growth of the plant under high concentrations of calcium sulphate. It is apparent that the addition of 0.02 and 0.05 per cent of calcium sulphate gave the most beneficial results.

TABLE VIII —*Effect of calcium sulphate on the growth of red clover in soil*

Treatment.	Number of nodules.	Average of group	Length of root.	Average of group.
	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
Untreated.....	9	10	6.5	7.2
Do.....	12		8.5	
Do.....	8		6.8	
Do.....	10		7.0	
Given 0.01 per cent of calcium sulphate.....	29	31	8.0	9.6
Do.....	33		10.0	
Do.....	11		10.5	
Do.....	51		9.0	
Given 0.02 per cent of calcium sulphate.....	34	33	8.0	9.5
Do.....	17		9.6	
Do.....	48		12.0	
Do.....	32		8.5	
Given 0.05 per cent of calcium sulphate.....	45	29	9.0	9.2
Do.....	36		6.5	
Do.....	18		11.3	
Do.....	19		11.5	
Given 0.1 per cent of calcium sulphate.....	13	14	7.0	7.6
Do.....	11		7.5	
Do.....	12		8.5	
Do.....	11		7.5	



The data in Table VIII show that calcium sulphate does increase the growth of the clover within a certain concentration. In amounts between 0.02 and 0.05 per cent it appears to be most beneficial. The results also show that calcium sulphate increases the root development and the number of nodules.

The effect of calcium sulphate on clover grown on Sparta acid sand was tested. Six kgm. of the sand admixed with 1 gm. of dipotassium phosphate were placed in each of ten 1-gallon jars. The composition of the Sparta acid sand used was as follows:

	Per cent.
Potassium.....	1.16
Nitrogen.....	.062
Phosphorus.....	.034
Organic matter.....	1.51

The jars were kept in the greenhouse and the moisture content held at 18 per cent. Each jar was seeded to red clover and then inoculated with a pure culture of red-clover organisms. After two weeks the jars were thinned to 20 plants in each. The plants grew luxuriantly, but there was no apparent difference in size until the sixth week. In jars 7 and 8, to which 0.05 per cent of calcium sulphate had been added, the increase in growth was considerable, while in jars 9 and 10, to which 0.1 per cent of calcium sulphate had been added, there was no appreciable increase. The jars to which 0.01 and 0.02 per cent of calcium sulphate had been added showed an increase in growth, but this increase was less than in jars 7 and 8. The green and dry weights of the clover were taken. The average weights of the clover are given in Table IX.

TABLE IX.—*Effect of calcium sulphate on red clover grown in Sparta acid sand*

Treatment.	Weight of crop.	
	Green.	Dry.
Untreated.....	Gm. 110.6	Gm. 19.4
Given .01 per cent of calcium sulphate.....	131.1	21.2
.02 per cent of calcium sulphate.....	146.7	21.7
.05 per cent of calcium sulphate.....	168.5	24.6
.10 per cent of calcium sulphate.....	145.8	17.5

These results show that calcium sulphate increases the growth of clover grown on Sparta acid sand. The increase, however, is confined to certain concentrations. The greatest increase was obtained at concentrations of 0.02 and 0.05 per cent.

#### EFFECT OF ELEMENTAL SULPHUR ON GROWTH OF RED CLOVER

For this experiment ten 1-gallon jars, each containing 6 kgm. of Miami silt-loam soil, were used. Various amounts of sulphur were added. The jars were kept in the greenhouse and the moisture content

held at 18 per cent. After four weeks these were seeded with red clover and inoculated with a pure culture of red-clover organisms. Two weeks later the number of plants was reduced to six per jar. There was no appreciable difference in the size of the plants until the fourth month. At this time those in the sulphur series showed an increase in growth. At the end of the fifth month this increase was more marked. The leaves of the plants in the jars to which 0.05 per cent of sulphur had been added were tinged with red at the edges. The stem also showed this red coloration, but to a lesser degree. At the end of the fifth month the tops were cut and weighed, green and dry, with the results shown in Table X.

TABLE X.—*Effect of elemental sulphur on the growth of red clover*

Treatment.	Weight of crop.	
	Green.	Dry.
	Gm.	Gm.
Untreated.....	25.3	6.25
Given .01 per cent of sulphur.....	32.6	6.90
.02 per cent of sulphur.....	29.4	6.75
.05 per cent of sulphur.....	30.8	6.80
.10 per cent of sulphur.....	34.0	7.00

The sulphur series showed a slight increase in yield. Several of the plants died, so that the number of plants in the various jars varied. The results therefore are not final. It seems safe, however, to say that sulphur increased slightly the yield of clover in Miami silt-loam soil. After the tops were cut the roots were carefully removed and washed. There was no apparent difference in the size or the number of nodules in the treated and the untreated series. All of the roots contained a great number of nodules.

## SUMMARY

(1) Calcium sulphate, when added to a soil, apparently has no marked effect on the total number of bacteria that grow on agar plates; nor does it produce any marked increase in ammonification or nitrification. This confirms the observations of Fred and Hart.<sup>1</sup>

(2) Large amounts of elemental sulphur cause a decrease in the total number of bacteria that grow on agar plates, but produce an increase in ammonification at concentrations of 0.05 per cent. This increase in ammonia is accompanied by a parallel decrease in nitrate formation. The decrease is very probably due to the acidity or toxicity produced by the oxidation of sulphur.

<sup>1</sup>Fred, E. B., and Hart, E. B. The comparative effect of phosphates and sulphates on soil bacteria. Wis. Agr. Exp. Sta. Research Bul. 35, p. 35-66, 6 fig. 1915.

(3) Calcium sulphate stimulates the growth of pure cultures of red-clover bacteria in nutrient solutions and in soil extract. The increase is as great with 0.01 per cent as with 0.1 per cent.

(4) The root development of red clover is increased by calcium sulphate, 0.01 per cent being apparently as efficient in producing this increase as 0.1 per cent.

(5) In small amounts calcium sulphate increases the yield of red clover and also the number of nodules. Concentration as high as 0.05 to 0.1 per cent, however, produces no increase in growth.

(6) The application of elemental sulphur to Miami silt-loam soil increased but slightly the yield of clover and apparently did not affect root development or nodule formation. In producing this slightly increased growth 0.01 per cent was as efficient as were higher concentrations.

(7) A review of the results of these experiments shows that calcium sulphate in soil does not produce any marked effect on the bacteria commonly found on agar plates, but does increase the growth of the legume bacteria. It also increases the yield of red clover, which is accompanied by a greater root development and a greater number of nodules.

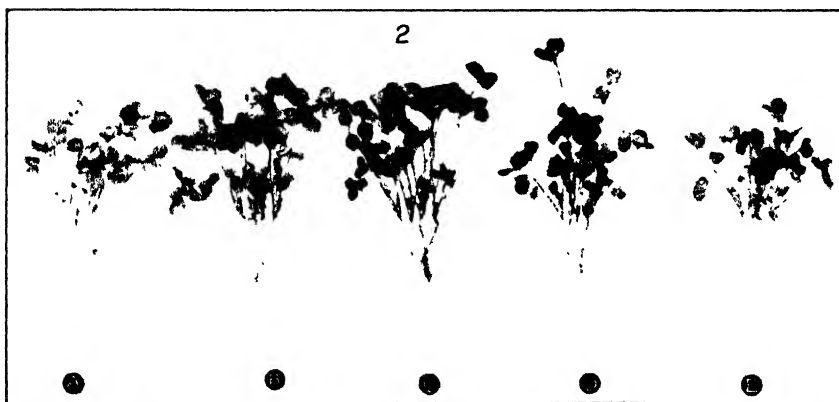
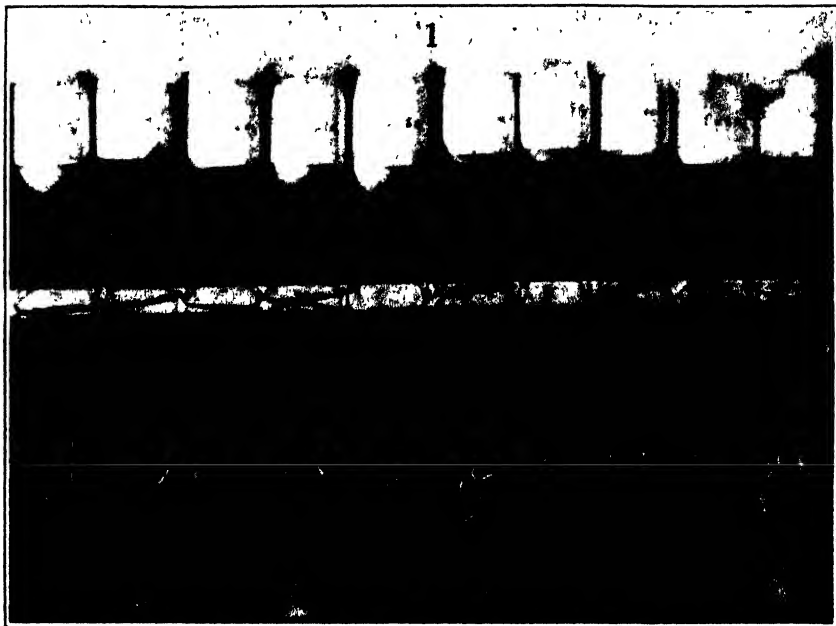
(8) The addition of sulphur increases the ammonification, but decreases nitrification and the total number of soil organisms. It increases the yield of red clover but slightly and does not affect the root development nor the number of nodules.



## PLATE LVI

Fig. 1.—Red-clover plants, showing the effect of treatment with calcium sulphate. The plants in these test tubes show the contrast in size of root between the treated and untreated tubes. The treated tubes were selected from various concentrations. Beginning at the left, tubes 1, 3, 5, 7, and 9 are untreated; tubes 2, 4, 6, 8, and 10 are of the calcium-sulphate series. Note the decided increase in length of root of the plants in the treated tubes as compared with those in the untreated.

Fig. 2.—Group A, untreated; B, 0.1 per cent of calcium sulphate added to Miami silt-loam soil; C, 0.02 per cent added; D, 0.05 per cent added; E, 0.1 per cent added.





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## A SERIOUS DISEASE IN FOREST NURSERIES CAUSED BY PERIDERMIIUM FILAMENTOSUM

By JAMES R. WEIR, *Forest Pathologist*, and ERNEST E. HUBERT, *Scientific Assistant, Investigations in Forest Pathology, Bureau of Plant Industry*

In June, 1914, several seedlings of *Pinus ponderosa* Laws., with the stems severely infected with a disease caused by a species of *Peridermium*, were received from the Savenac nursery of the United States Forest Service, at Haugan, Mont. The seedlings were taken from the field-planting area located near the nursery. They had remained one year in the seed beds, one year in the transplant beds, and two years in the field. It seemed likely that the seedlings became infected while in the nursery, since the few yellow pines in the near vicinity of the area were free from the fungus.

On July 2, 1914, *Castilleja miniata* Dougl., growing in abundance on the nursery site, was found bearing the fungus *Cronartium coleosporioides* (D. and H.) Arthur.<sup>1</sup> No other species of *Cronartium* was found. Evidence of the æcial stage on left-over yellow-pine seedlings in the transplant beds brought the two stages in such close proximity it seemed certain that the fungus on the pine seedlings could be no other than *Peridermium filamentosum* Peck. Since the Savenac nursery has an annual output of 1,600,000 yellow-pine seedlings, it was evident that measures should be employed immediately to prevent the spread of the disease.

On May 1, 1915, all of the 2-year-old yellow-pine seedling beds were found to be infected with the fungus. The seedlings were being prepared for shipment to the planting areas in the forests, and a thorough inspection was made of all the bundled stock. All visibly infected seedlings were removed and burned. The seedlings remaining in the beds were examined, and the infected ones similarly destroyed. More than 4 per cent of the plants gave outward evidence of being attacked. Of the 10,000 seedlings inspected 432 were removed and burned. Control

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<sup>1</sup> Meinecke, E. P. Notes on *Cronartium coleosporioides* Arthur and *Cronartium filamentosum*. *In* *Phytopathology*, v. 3, no. 3, p. 167-168. 1913.



methods were devised and recommended, and, as the bundling of seedlings progressed, all visibly infected trees were removed and burned. A sharp watch was kept on the beds to remove new infections as they developed.

Most of the infections were found along the north and east borders of the seedling beds. A large patch of *Castilleja miniata* was growing on the edge of a lodgepole pine (*Pinus murrayana* "Oreg. Com.") stand near the creek bank directly northeast of the infected seedling beds and not more than 200 feet distant. The records of the weather station located on the grounds show that the prevailing winds blow both northeast and southwest, which is an important factor in spore distribution between the two hosts. Thus, these winds sweep northeast over the patch of *Castilleja miniata* from the 2-year-old yellow-pine seedlings and in reversing blow from the former to the latter. In this manner the æciospores from the infected yellow pine are distributed to the castilleja plants and the sporidia borne on the castilleja leaves are transmitted to the young trees in the beds. On May 13, 1915, this fungus infection was found to be of serious importance on the yellow pine.

From fresh specimens of the blister rust brought in to the greenhouse at Missoula, Mont., two plants of *Castilleja miniata* were inoculated on May 3, 1915. These were covered with oiled-paper bags and labeled. Six control plants of the same species were potted and bagged and kept in a separate part of the greenhouse. On May 23 uredospores developed on the underside of the leaves of the two inoculated plants, while the control plants remained normal. Later the teliospores developed, sporidia being produced on May 29. Duplicate experiments were conducted at the field camp at Priest River, Idaho. Æciospores from the infected yellow-pine seedlings were sown on *Castilleja miniata* on May 14, and they gave positive results on June 11. The characteristic filamentous structure of the æcia on the pine seedlings and these transfers of the fungus to castilleja prove the fungus to be *Peridermium filamentosum* Peck.

On May 13, 1915, the native lodgepole pine surrounding the nursery was found to be infected with a trunk, a branch, and a needle form of *Peridermium*. The structure of the æcia of these forms indicated that the trunk and the branch forms were identical. The trunk form (known locally as the "hip canker" of the lodgepole pine) and the branch-gall form in the Rocky Mountain region have been commonly united under the name "*Peridermium harknessii* Moore."<sup>1</sup> Later they were transferred to *Peridermium cerebrum* Peck by Arthur and Kern.<sup>2</sup>

The following inoculations, made recently at Missoula, Mont., by the writers, prove that the "hip canker" and the gall-forming *Peridermium* of the lodgepole pine are both *Peridermium filamentosum*.

<sup>1</sup> Harkness, H. W. New species of California fungi. In *Bul. Cal. Acad. Sci.*, v. 1, no. 1, p. 37. 1884.

<sup>2</sup> Arthur, J. C., and Kern, F. D. North American species of *Peridermium* on pine. In *Mycologia*, v. 6, no. 2, p. 133-138. 1914.

On May 17, 1915, æciospores from the "hip canker" of *Pinus contorta* from Haugan, Mont., were sown on two plants of *Castilleja miniata* under control conditions in the greenhouse at Missoula. On June 3 uredospores were present on the leaves. The teliospores appeared June 14. The two control plants remained healthy. The Cronartium was identical with that previously produced by the inoculations on *Castilleja miniata* with æciospores from the *Peridermium* on the 2-year-old seedlings of *Pinus ponderosa*. This demonstrates the identity of the "hip canker" *Peridermium* with *Peridermium filamentosum*.

The following cultural data show that the gall-forming *Peridermium* of the lodgepole pine is likewise identical with *Peridermium filamentosum*. On May 25, 1915, æciospores from the gall-forming *Peridermium* on branches of lodgepole pine were sown by the writers on three plants of *Castilleja miniata* under control conditions in the greenhouse. By June 11, 1915, uredospores had developed on the leaves, telia and sporidia being produced 10 days later. The two control plants remained healthy.

Check experiments carried on at the field camp at Priest River, Idaho, gave similar positive results. Six plants of *Castilleja miniata* were inoculated and gave positive results. All three control plants remained healthy.

Cultures, under control, made both in the greenhouse and in the field, on *Castilleja miniata* with æciospores taken from the blister rust on the lodgepole pine commonly known as *Peridermium stalactiforme* A. and K., have produced *Cronartium coleosporioides* (D. and H.) Arthur. Two plants of *Castilleja miniata* were inoculated and two control plants set aside. Both inoculated and control plants were covered with oiled-paper bags. The inoculated plants gave positive results and the controls remained healthy. This confirms the results of Meinecke<sup>1</sup> and the conclusions of Arthur and Kern<sup>2</sup> and places *Peridermium stalactiforme* without further doubt under *Peridermium filamentosum*.

The absence of oaks (*Quercus* spp.), the alternate hosts of *Peridermium harknessii*<sup>3</sup> and *Peridermium cerebrum*, from this region where the species of *Peridermium* on the lodgepole pine is so prolific, the characteristic filamentous processes in the æcia of the various forms of *Peridermium* appearing on the lodgepole pine, and the inoculation experiments successfully conducted on *Castilleja miniata*, all exclude the possibility of this fungus being other than *Peridermium filamentosum*.

The yellow-pine seedlings in the nursery were free from traumatic injuries. This is explained by the fact that they had remained in the same bed since germination and thus were not exposed to the injury from transplanting. All seedlings showing slight corrugations or blisterings of the lower stems gave no evidence of mechanical injury, but they

<sup>1</sup> Meinecke, E. P. Op. cit.

<sup>2</sup> Arthur, J. C., and Kern, F. D. Op. cit.

<sup>3</sup> Hedgcock, G. G. Notes on some western Urediniae which attack forest trees. II. In *Phytopathology*, v. 3, no. 1, p. 15-17. 1913.

developed the bright orange eruptions of the rust later. It is safe to draw the conclusion that the spore tubes which produce the infections in the seedlings penetrate the host in the absence of all surface openings due to the mechanical injuries. The period of development between the time of penetration of the host and the appearance of the æcial eruptions on the stems is about 10 to 11 months. The seedlings in question were produced from seed sown in the spring of 1913, and the spring of 1914 some of the seedlings produced the æcial eruptions. The seedlings must have been infected in the period following germination and have developed the fruiting stage in the spring of the following year. The infecting spores could have been either sporidia from the species of *Cronartium* on *Castilleja miniata* or possibly æciospores from the surrounding lodgepole pines infected with *Peridermium filamentosum*. Facultative autoecism in *Peridermium filamentosum* is as yet not proved, but it is suspected of being a "repeater."

During the period from May 29 to June 2, 1915, Mr. E. C. Rogers, of the Forest Service nursery at Haugan, Mont., assisted in the work of visiting and inspecting the various plantation areas near Wallace, Idaho, on the Coeur d'Alene National Forest, and those in the vicinity of Savenac nursery and Deborgia, Mont., on the Lolo National Forest. In all an area of approximately 500 acres was covered. The inspection was confined principally to the yellow-pine plots, with particular attention to the plants taken from the infected 2-year-old yellow-pine beds at Savenac nursery. Very few infections caused by species of *Peridermium* were recorded, some of the areas being entirely free from visible signs of the rust, although it may be present and not appear until the following year or later. In the case of the "2-year-old yellow pine, unfertilized" plot, which was planted in the spring of 1915, the few infections observed were found to be covered by the moist earth because of deep planting and thus were rendered practically incapable of spreading. Two of these infections were molded and the spores were no longer viable. The Placer Creek area near Wallace, Idaho, is a clean-burn site, the fires of 1910 having destroyed all living timber. No living pines or *castilleja* plants are to be found growing within a considerable distance of this area. *Castilleja miniata* and *Pinus contorta* are plentiful in the area containing 4-year-old yellow-pine seedlings located on the ridge west of the Savenac nursery. Very little visible infection was found on this plot. These facts prove the effectiveness of the inspection work in checking the spread of the disease and the necessity for culling out and burning the infected seedlings as soon as the eruptions make their appearance.

On June 1, 1915, a survey was made of the area surrounding the nursery beds for a distance of half a mile. Fifty per cent of the lodgepole-pine stand in close proximity to the beds was badly infected with *Peridermium filamentosum*. A group of 61 trees, having diameters (breast

high) of 5 inches and over and growing within 100 feet of the nursery beds, was found to be very seriously infected. Of the 61 trees, 26 had large cankers encircling the trunks varying in length from 2 to 8 feet. The branches and twigs were infected. *Peridermium montanum* was also present on the needles. *Castilleja miniata* was found growing in abundance under the trees. Lodgepole-pine seedlings in and near this area were, with rare exceptions, heavily infected with the twig and stem and the needle forms of *Peridermium*. Very little native yellow pine was found growing in the vicinity, most of the trees having been killed by the fires of 1910. A few veteran trees remain growing upon the ridge west of the nursery, but these show no evidence of fresh eruptions of *Peridermium*. These facts point to the lodgepole pine as the original distributor of infection to the yellow-pine seedling beds in the nursery.

Experiments are being conducted in an effort to control the disease. The seedlings in the nursery beds are being sprayed during the infection period. An effort is being made to eradicate the alternate host from the vicinity by mechanical or chemical means. The felling and burning of trees near by infected with *Peridermium* will reduce the chances of infection. The possibility of the fungus possessing facultative autoecism, the close proximity and abundance of the alternate host, and the prolific development of the same fungus upon lodgepole pine in the vicinity of the seedling beds all make *Peridermium filamentosum* a dangerous enemy to deal with in this nursery and one to be reckoned with in other forest nurseries where similar conditions exist.

#### SUMMARY

*Peridermium filamentosum* Peck has been found to cause a serious disease of yellow-pine seedlings at the Savenac nursery located at Haugan, Mont.

The various forms of *Peridermium* occurring on lodgepole pine at this nursery, with the exception of the foliicolous species, have been demonstrated to be *Peridermium filamentosum*, having an alternate stage on species of *Castilleja*.

The fact that the same species of *Peridermium* attacks both the lodgepole pine and the yellow pine increases the difficulty of control of this fungus.

The proximity and abundance of the alternate host (*Castilleja miniata*) of *Peridermium filamentosum* and its prolific development on lodgepole pine in the vicinity of the seedling beds tend to make this disease a dangerous one in forest nurseries.



# SWEET-POTATO SCURF

By L. L. HARTER,

*Pathologist, Office of Cotton and Truck Disease Investigations,  
Bureau of Plant Industry*

## INTRODUCTION

The scurf disease of the sweet potato (*Ipomoea batatas*) was first described by Halsted,<sup>1</sup> who published a brief account of it in 1890. To the fungus he gave the name *Monilochaetes infuscans*, a new genus and species, of which, unfortunately, he gave no technical description. For many years following his pioneer work little or no attention was given to sweet-potato diseases. This very common and interesting disease was therefore passed over until a few years ago, when the writer and others took up a study of them. For almost five years the disease has been under observation and study. It is therefore for the purpose of completing the description of the organism and recording the results of inoculation experiments and certain characteristics of the fungus heretofore unpublished that this paper is prepared.

## GENERAL APPEARANCE OF THE DISEASE

Scurf is characterized by a brown discoloration of the surface of the underground parts of the sweet potato (Pl. LVII). The discolored areas may occur as spots of varying size and shape, with no definite outline, or as a uniform rusting of the entire surface. In gross appearance it reminds one somewhat of the silver scurf of the Irish potato, although it is somewhat darker. However, it does not penetrate the host to the extent that silver scurf does. The scurf of the sweet potato produces no rupture of the epidermis and is so superficial as to be easily scraped off by the finger nail.

## DISTRIBUTION, PREVALENCE, AND LOSS

The writer has found the scurf very prevalent on sweet potatoes in New Jersey, Delaware, Maryland, Virginia, North Carolina, Ohio, Illinois, Iowa, and Kansas, and to a slight extent in other States. The following varieties are susceptible to scurf in varying degrees: Eclipse Sugar Yam, General Grant Vineless, Florida, Nancy Hall, Yellow Yam, Miles Yam, Red Brazilian, Dahomey, Yellow Strasburg, Pierson, Key West Yam, Vineless Yam, Southern Queen, Big Stem Jersey, Yellow Jersey, and Early Carolina. It is probable that the disease occurs on other varieties as well.

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<sup>1</sup> Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Exp. Sta. Bul. 76, p. 25-27, fig. 17. 1890.

Scurf is more prevalent in heavy, black soils and in soils that have been heavily manured or contain a larger amount of organic matter than in light, sandy soils.

The loss to the crop caused by the scurf is perhaps small in comparison with that caused by some of the more virulent diseases. Nevertheless, the actual financial loss throughout the country that can be attributed to this disease alone amounts to considerable. Scurfy potatoes do not command as high a price in the markets as clean ones, though if otherwise sound they are just as good for consumption. The fungus under favorable conditions, such as a relatively high humidity and temperature, continues to develop under storage conditions to a limited degree. It weakens the host, so that during periods when the storage house is rather dry the potato loses moisture and becomes shriveled and dried, rendering it unfit for sale and at the same time less resistant to the attacks of other parasites. Taubenhaus<sup>1</sup> claims that the fungus on the potato is easily killed by immersing for 10 minutes in a solution of mercuric chlorid (1:1,000).

#### ISOLATION OF THE FUNGUS

Some difficulty was experienced at first in isolating the fungus, since it proved to be a very slow grower and developed but little or not at all on some kinds of media. After some experimentation with different media it was found to make a slow growth in Irish-potato, string-bean, and oatmeal agar. By thoroughly washing the potato and disinfecting for about one minute in a solution of mercuric chlorid (1:1,000) and planting bits of the tissue in plates of oatmeal agar by means of sterile instruments a pure culture could generally be secured. In a week or 10 days transfers were made to media in test tubes, usually cooked rice in water or sterile, moistened corn meal. At the end of three or four weeks on these media a matted growth of dark-brown hyphæ developed. Hyaline spores are produced in abundance on long, stout conidiophores in tubes of cooked rice.

#### INOCULATION EXPERIMENTS

Inoculation experiments were begun on October 13, 1914, and performed as follows: Sound potatoes were thoroughly washed in water and placed in moist chambers with moistened filter paper in the bottom. They were then sprayed with a suspension of spores and bits of broken hyphæ of the scurf fungus in sterile water and exposed to laboratory room conditions. Water was added from time to time, as necessity required, to maintain the humidity of the moist chamber. At the end of two weeks small centers of infection appeared indiscriminately over the surface of the potatoes. These centers gradually enlarged, either by the merging of two or more spots or by the enlargement from a single center. There is undoubtedly considerable enlarging of the spots in moist chambers from

<sup>1</sup> Taubenhaus, J. J. Soil stain and pox, two little known diseases of the sweet potato. (Abstract.) *In* *Phytopathology*, v. 4, no. 6, p. 405. 1914.

centers of infection, in view of the fact that conidiophores often  $200\mu$  in length stand erect or at an angle on the surface of the potato and drop their spores, starting new infections outside the point of original growth. The spots, however, so far as the writer has been able to determine, do not enlarge by the branching and creeping of the hyphæ over the surface. Repeated inoculation experiments gave similar results. The checks remained free from the disease.

#### DESCRIPTION OF THE FUNGUS

The young vegetative growth of *Monilochaetes infuscans* is hyaline and septate. At the end of a few days, however, with the exception of the terminal cell of the conidiophore, the hyphæ turn densely brown. On the host little or no branching of the vegetative growth takes place. Although Halsted figured a branching of the hyphæ which was hyaline in color within the tissues of the host, the writer, after long and detailed examination of paraffine sections and sections prepared in other ways, has not been able to find a sure example. The sporophores, for such they appear to be, arise from the surface of the host and are attached to it by an enlarged end cell slightly buried in the cuticle (Pl. LVIII, E, C, D). Occasionally a second (Pl. LVIII, I) or third (Pl. LVIII, J) enlargement or bulblike growth is found deeper in the host or parallel with the surface (Pl. LVIII, G). From some of these secondary enlargements a conidiophore may be developed (Pl. LVIII, F, H). Plate LVIII, E, C, shows conidiophores bearing conidia produced on the host. The brown septate conidiophores vary in length from 40 to  $175\mu$  and bear at the end a single-celled spore, which on the host is slightly brown or hyaline. The conidia are 12 to  $20\mu$  in length by 4 to  $7\mu$  in thickness.

This fungus, as might be expected, behaves differently when grown artificially. Growth has been carefully observed on a few of the common media—namely, Irish-potato agar, beef agar, rice agar, oatmeal agar, string-bean agar, Irish-potato cylinders, sweet-potato stems, and stems of *Melilotus alba*. At the end of 24 days a very slight growth appeared on string-bean agar, rice agar, and oatmeal agar at a temperature varying from  $6^{\circ}$  to  $7^{\circ}$  C. Conidia were very sparingly produced. At room temperature ( $23^{\circ}$  to  $26^{\circ}$ ) growth was visible on all media in 4 days, except on rice agar and the stems of sweet potatoes and *Melilotus alba*. In 13 days a small growth appeared on rice agar, but on stems of sweet potatoes and sweet clover no growth was detected at the end of 4 weeks. There is very little difference in the gross appearance of the growth on any of the media used. Enlargement from a single center is very slow, attaining a diameter of about 2 to 5 mm. in 14 days. The fungus piles up in an almost black feltlike mass 2 to 3 mm. in height, with an entire margin. It penetrates the medium but little. The vegetative hyphæ in mass are almost charcoal-black, although in gross appearance there is some variation on different culture media. On Irish-



potato cylinders and Irish-potato agar the growth has a darker appearance than on oatmeal agar, beef agar, and string-bean agar, owing to the fact that the numerous erect conidiophores bearing hyaline spores are produced in greater abundance on the three latter media and give a grayish appearance to the upper surface. If the conidiophores and spores be scraped away, the mass is black beneath. Growth appeared only on oatmeal agar at temperatures varying from 30° to 32° in 14 days. From these results it appears that temperatures as low as 6° to 7° and as high as 30° to 32° prohibit the normal growth of the fungus.

The vegetative growth on artificial cultures is hyaline at first and later brown (Pl. LVIII, L), with the exception of the end cell of the conidiophore, which at its outer extremity is hyaline to slightly brown (Pl. LVIII, A, B, L). The conidiophores are branched, septate (Pl. LVIII, A, L), and vary in length from 30 to 225 $\mu$ . The conidia are continuous, granular, and hyaline to slightly brown with age (Pl. LVIII, M). As soon as one conidium is mature, it separates easily from the conidiophore and another begins growth by a swelling of the end cell of the conidiophore, to be dropped in turn when mature. This process is repeated as long as the environment of the host will permit. It should be noted in this connection also that this fungus can be reproduced by hyphæ as well as from the spores. It is likely also that vegetative reproduction accounts for a larger part of the infections under natural conditions. In fact, certain vegetative parts might be confused with or mistaken for conidia. Although conidia are not produced in abundance on the host, they frequently develop normally on diseased potatoes kept for some days in a moist chamber.

The conidia under laboratory conditions germinate slowly in rice or sweet-potato decoction. One or two growths (Pl. LVIII, K) are thrown out usually at the end of the conidia, which attain in 24 hours a length about equal to that of the spore. The branching of the hyphæ begins the second day (Pl. LVIII, N), and the production of the brown pigment in about three days.

#### TAXONOMY OF THE FUNGUS

Halstead attributed the scurf to a new genus and species, *Monilochaetes infuscans*, but he gave no technical description of it that the writer has been able to find. The fungus belongs to the Dematiaceae of the Hyphomycetes. However, the writer has been unable, after considerable study of the fungus, to fit it into any of the genera so far described. It is, however, desirable, in view of the fact that it is a rather common and conspicuous fungus, that it have a description by which it may be recognized. The fungus has been known as *Monilochaetes infuscans* and as the cause of the sweet-potato scurf for 25 years. Taubenhau and Manns<sup>1</sup> in a recent publication likewise refer to *Monilochaetes infuscans*

<sup>1</sup> Taubenhau, J. J., and Manns, T. F. The diseases of the sweet potato and their control. Del. Agr. Exp. Sta. Bul. 209, p. 11. 1915.

as the cause of the disease. In view of these facts, it is believed preferable to give it a description and permit it to maintain generic rank rather than to place it in a genus where it does not naturally belong.<sup>1</sup>

### MONILOCHAETES

Hyphae dark, erect, rigid, septate, not in definite fascicles; conidia distinctly different from the sporophores and hyphae, hyaline, slightly brown with age, continuous, not in chains, acrogenous.

### *Monilochaetes infuscans*

On the host definite vegetative hyphae are lacking; sporophores septate, erect, unbranched, dark, and attached to the host singly or by twos, by a bulblike enlargement 40 to 175 $\mu$  long, 4 to 6 $\mu$  wide, bearing rarely a hyaline one-celled oblong spore. In cooked rice the hyphae are much branched, septate, brown; sporophores brown except at terminal cell, which is frequently hyaline to slightly brown, septate, branched, stout, 30 to 225 by 4 to 6 $\mu$ ; conidia abundant, one-celled, hyaline, ovoid to oblong, 12 to 20 by 4 to 7 $\mu$ , solitary, terminal.

Parasitic on the underground parts of *Ipomoea batatas*. Type specimens deposited in the pathological collection of the herbarium of the United States Department of Agriculture, Washington, D. C.

### SUMMARY

The scurf disease of the sweet potato was first recognized in 1890 by Halsted, who named the fungus "*Monilochaetes infuscans*," a new genus and species. He failed, however, to describe either the genus or species. The scurf has been found prevalent in nine States and sparingly in others, and on 16 varieties of sweet potatoes. The organism has been shown by inoculation experiments to be the true cause of the disease. A detailed discussion of the morphology of the organism is taken up, also its growth on different culture media at different temperatures. It was found that the organism on the host consisted merely of sporophores and conidia. In culture, however, well-defined branched mycelia and spores developed.

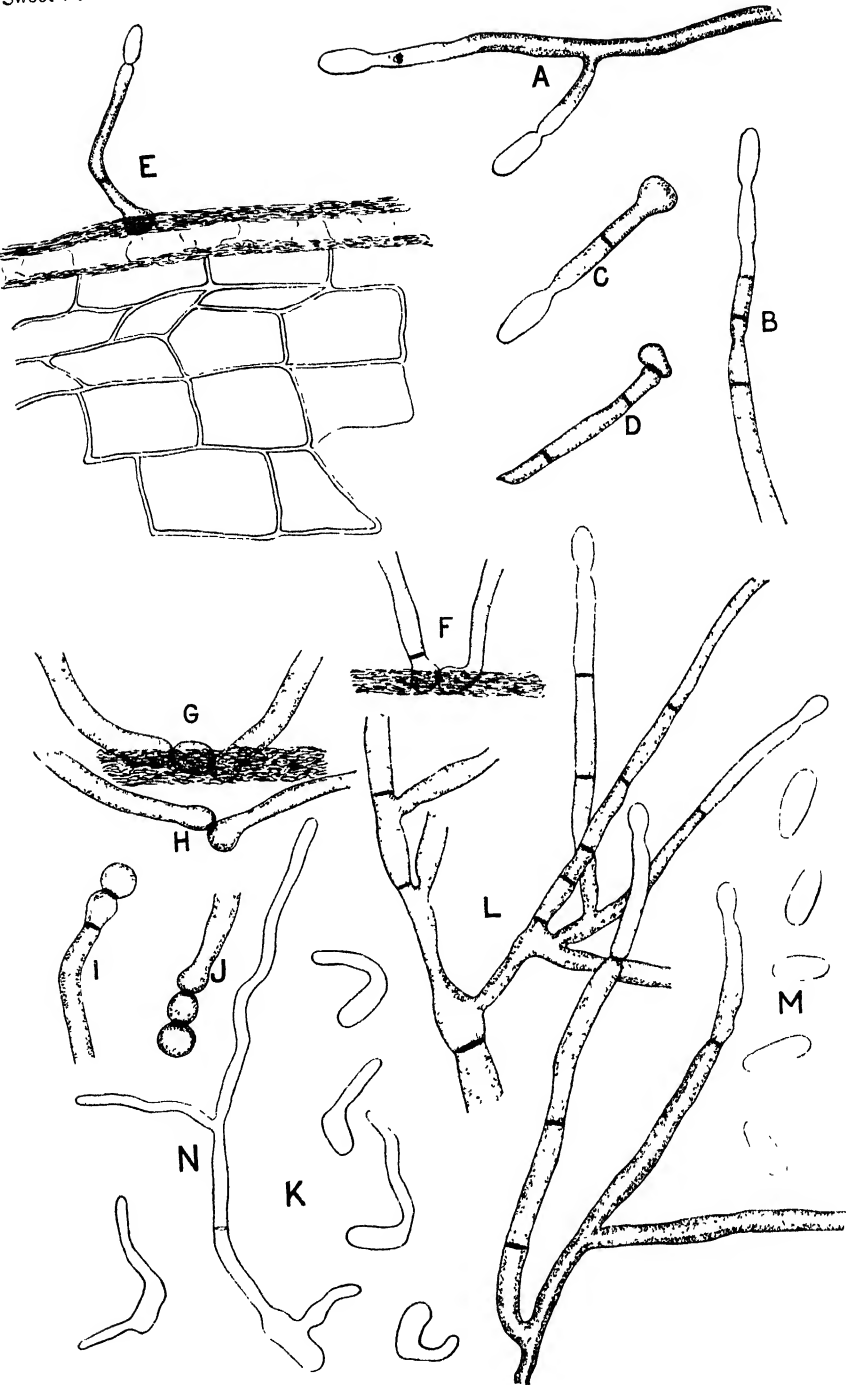
<sup>1</sup> The writer is indebted to Dr. C. L. Shear and Mrs. Flora W. Patterson, of the Bureau of Plant Industry, for having examined specimens of this fungus.

**PLATE LVII**

**A sweet potato showing the discoloration produced by *Monilochaetes infuscans*.**

**(792)**





## PLATE LVIII

### *Monilochaetes infuscans:*

*A*, a branched conidiophore with conidia attached. *B*, an unbranched conidiophore, showing septation; conidium attached. *C*, a conidiophore from host, with conidium attached. *D*, a conidiophore from the host, showing the peculiar basal cell and septation. *E*, a conidiophore bearing conidium, showing diagrammatically the attachment to the host by a bulblike enlargement of the basal cell. *F*, two conidiophores joined at the base and slightly sunken in the tissue of the host. *G*, two conidiophores joined by a single oblong cell. *H*, two conidiophores joined at the base and slightly sunken in the tissue of the host. *I*, a conidiophore from the host with an almost spherical cell attached to the enlarged end cell. *J*, a conidiophore, showing an attachment of two almost round cells to the enlarged basal cell. *K*, germination and growth of conidia in a sweet-potato decoction in 24 hours. *L*, hyphæ from a culture, showing characteristic branching and septation. *M*, a group of mature conidia. *N*, germination, growth, branching, and septation of the fungus at the end of 42 hours in a sweet-potato decoction.

*E* is drawn to a scale of 200; all others to a scale of 500.



# BANANA AS A HOST FRUIT OF THE MEDITERRANEAN FRUIT FLY

By E. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant*,  
*Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

## INTRODUCTION

The banana export trade of the Hawaiian Islands amounted to 256,319 bunches of Chinese bananas (*Musa cavendishii*) during the year ending June 30, 1915. Although 25,448 bunches were shipped during June, 1915, the monthly average for the year was 19,621. With such a trade with the California coast established, it became imperative to determine to what extent bananas are infested by the Mediterranean fruit fly (*Ceratilis capitata* Wied.), in order that data might be placed on file for the guidance of the Federal Horticultural Board in forming its quarantine regulations for the protection of mainland fruit interests. While it has been proved that bananas may serve as host fruits of this fruit fly when ripe, all data happily corroborate the general belief among shippers and growers, as well as among entomologists familiar with the situation, that Chinese bananas and Jamaica or Bluefield bananas (*Musa* spp.), when cut and shipped under commercial conditions, are immune to attack and offer no danger as carriers of this pest if properly inspected and certified as provided for by the regulations of the Federal Horticultural Board (8).<sup>1</sup> These regulations, it may be stated, provide for inspection in the packing sheds for the presence of prematurely ripe, bruised, cracked, and decayed fruits; require the use of safe packing material; and prohibit the shipment of bananas from plantations the surroundings of which have not been favorably passed upon from a fruit-fly standpoint by a representative of the Board.

## EVIDENCE FROM TRAPS AS TO THE PRESENCE OF ADULT FRUIT FLIES IN BANANA PLANTATIONS

The establishment of a series of traps among banana plants has shown that adult fruit flies are everywhere present in banana plantations in Hawaii. Traps were placed in the Moanalua, Moiliili, Waikiki, Mokuleia, Kawaihapai, and Puuiki plantations. As many as 793 adult flies were taken in one trap suspended from a bunch of bananas in a field at Moanalua between July 28 and August 7, 1913. Traps hung in the much larger and exceptionally well isolated banana fields of Puuiki, Kawaihapai, and Mokuleia in the Waialua district of Oahu showed a

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<sup>1</sup> Numbers in parentheses refer to "Literature cited," p. 803.



far smaller number of adults, yet a sufficient number to infest bananas were they readily subject to infestation. In this district 57 traps caught no flies between August 9 and 21, 1913, while the average for the same period for 119 traps in which flies were caught amounted to 7.5 adults. Flies were taken in all traps hung at Moanalua, Waikiki, and Moiliili, although some of the traps were hung in the center of the largest blocks of trees. At Moanalua as few as 22 and as many as 3,334 adult flies were taken from individual traps between July 15 and August 29, 1913, while at Waikiki and Moiliili as few as 1 and as many as 402 adults were taken between June 17 and July 8, 1913. Thirty-six was the largest number of flies taken from any trap at Waialua between August 9 and 21, 1913. Although only males were caught in the traps, adults caught in the hand net showed the sexes to be present in the usual proportion among the banana plants. These data determine at once the fact that the general immunity of bananas is not due to any lack among banana plants of adult fruit flies capable of ovipositing.

#### ABSENCE OF INFESTATION AMONG RIPE AND GREEN BANANAS, AS EVIDENCED BY FIELD INSPECTIONS AND LABORATORY REARINGS

During the period of somewhat over three years that the Federal Government has had supervision of the inspection of export bananas in the Hawaiian Islands (from August, 1912, to the present time) the writers have seen no case of infestation among ripe or green bananas grown under normal field conditions, and neither have the banana inspectors. Frequently individual fruits on a bunch of bananas will ripen in advance of the other fruits. When the bunches are cut, these prematurely ripe fruits, which often in addition have the peel split so as to expose the pulp, are removed before shipment and discarded at the packing sheds. If any bananas are subject to infestation, it would seem that these fruits are most likely to be; yet 1,044 prematurely ripened fruits brought to the laboratory during 1913 and 1914 and placed in rearing jars yielded no adult flies, although they came from fields known to harbor adult flies. During August, 1914, when large numbers of flies were maturing from peaches in a garden in Manoa Valley, fully ripe Chinese bananas, and a variety known to the Hawaiians as the apple-banana (*Musa* sp.), growing in the midst of other species of infested fruits, showed no infestation. Thirty-nine fully ripe apple-bananas grown near the insectary from which flies were continually emerging showed no infestation. An examination of 27,000 fruits of the Chinese banana ready for shipment at several banana fields at Moanalua during early July, 1913, when records showed the adult flies to be very abundant, failed to reveal a single distinct egg puncture. Even suspicious abrasions were investigated and found not to extend through the skin nor to contain fruit-fly eggs. An examination of 3,500 similar fruits at Kalauao during July, 1913, also gave negative results. No fruit flies have been reared from about 1,000 green Chinese bananas

discarded at time of shipment at the packing sheds because of split peelings or black decayed ends. Fifty fruits of the Hawaiian variety, known as the "ice-cream" banana (*Musa* sp.), cut from the tree as they were turning color, showed no infestation, though growing in the midst of other species of infested fruits. No infestation was found among 500 overripe fruits of the Manila Hemp banana (*Musa textilis*) growing near the corner of King and Punabon Streets, Honolulu, nor among 60 fruits of the Borabora banana (*Musa fehi*), known to the Hawaiians as the Polapola banana, in a ripe though not soft condition, growing in a mountainous ravine at the head of Manoa Valley, Oahu.

There are no records of infestation of the Chinese and Bluefield bananas grown under commercial conditions in the Hawaiian Islands, or developing and ripening in city lots.

#### INFESTATION OF POPOULU AND MOA VARIETIES

The only case of infestation among bananas growing in the field was brought to the attention of Mr. David Haughs, of the Territorial Board of Agriculture and Forestry, on October 17, 1913. The infested fruits were of the Popoulu and Moa varieties (2) of the Popoulu group (*Musa* spp.) of cooking bananas. These are short, thick bananas, with comparatively thin skins. They are never eaten raw and, unlike the Chinese or Bluefield bananas, are rarely, if ever, shipped from the islands. They are very scarce and are strikingly distinct both from the ordinary cooking banana and from the banana of commerce.

Of the 11 fruits on the bunch of Popoulu bananas when the examination was made 7 were still green, though on the point of turning yellow, and 4 had turned yellow. There were in the peel no splits nor mechanical injuries and there was every evidence that the punctures found in three of the four ripe fruits had been made while the bunch was still on the tree. Mr. J. C. Bridwell, of the Hawaiian Board of Agriculture, had charge of the rearing, but kept no definite record of the number of adult flies reared from infested fruits. That larvæ matured and emerged from one fruit at least is evidenced by the numerous emergence holes in the peel (Pl. LIX, fig. 1).

The Moa variety was growing in the same garden with the Popoulu banana. The fruits of this variety are much larger and the peel thicker. Of 9 fruits taken from the single bunch found, 5 were perfect, but the peel of the 4 other fruits was so cracked that the pulp was well exposed; all were green in color but mature and about to turn yellow. Mr. Bridwell's notes, which have been placed at the writers' disposal through the courtesy of the Territorial authorities, state that of 12 distinct attempts at oviposition made in the peel of the 4 sound fruits, only one puncture was sufficiently deep to contain eggs, but no eggs were deposited. Only one of the 4 cracked fruits developed larvæ, and the eggs from which

these hatched were laid directly into the pulp along the crack in the peel. Of the punctures found in the peel of the cracked fruits, only one contained eggs, and these were dead and shriveled. Mr. Bridwell kept no definite record of the number of adult flies reared, but it was large. He estimates that from the Popoulu and the Moa fruits he reared about 350 adults. The thoroughness with which the larvæ destroyed the pulp of the Moa banana is shown in Plate LIX, figure 2.

Special attention should be called to the fact that infestation of the pulp in these two varieties occurred only in the fully ripe and yellow fruits of the Popoulu variety, which has a very thin skin, and in the fruits of the Moa variety, the peel of which was cracked, thus removing from the exposed pulp beneath the natural barrier to infestation referred to below. The ordinary cooking bananas, such as are in general use in the islands, are quite unlike the Popoulu and Moa varieties in shape.

#### EXPERIMENTS TO FORCE INFESTATION

While infestation of Hawaiian bananas has never been known to occur among fruits grown and harvested in accordance with trade requirements and prepared for shipment in accordance with the regulations of the Federal Horticultural Board, experiments have been carried on under more or less artificial and abnormal conditions for the purpose of determining whether the general immunity of commercially grown bananas in Hawaii is due to the presence of other host fruits for which the fruit fly has a greater preference or to some characteristic which renders them actually immune. Such experiments have been completed both in the field and in the laboratory.

#### EXPERIMENTS IN THE FIELD

As the writers have found that in the field they can bring about an infestation of ripe bananas, or in the laboratory of green but well-grown bananas that have been cut from the tree so long that the protecting sap has ceased to flow to any extent, their field experiments have been confined mainly to forcing, if possible, an infestation of bananas still attached to the tree yet sufficiently mature for the export trade.

During March, 1913, a rearing cage, 9 by 15 by 24 feet, was built over 20 Chinese banana trees bearing 14 bunches of bananas. Into this cheese-cloth-covered cage (Pl. LXII, fig. 1, 2) were introduced from time to time a total of over 3,000 Mediterranean fruit flies. The foliage within the cage was sprayed every few days with a solution of pineapple juice and water, as there was nothing else upon which the fruit flies could feed. As the fruits on the various bunches ripened, they were cut and placed in rearing jars in the insectary. The 14 bunches represented approximately 1,000 fruits, which ripened over a period extending from the middle of March to June 28. No adult flies developed from any of this fruit.

In order more closely to confine gravid females with bananas ripe enough for shipment, a fine wire cylinder, 20 inches in diameter and

30 inches long, closed at each end by cheesecloth, was placed over the entire bunch. From 200 to 500 fruit flies were introduced through the lower opening and allowed to remain with the fruit from 24 to 48 hours. The cage was then removed, the bunch cut, and the individual fruits examined for evidences of oviposition. Out of a total of 1,449 fruits thus carefully examined, 1,363 showed no evidence of attempted oviposition, while 86 bore puncture marks. In the peel of these 86 fruits the females had made 169 breaks in attempts to oviposit. Only two punctures were sufficiently deep to permit oviposition, and of these only one contained a single egg. This egg was deposited between August 21 and August 23, 1913, and by August 27, when the examination was made, fully two days after the egg should have hatched under normal conditions, it was found dead and blackened. None of the other attempts at oviposition extended for more than one thirty-second of an inch below the surface, while nearly all were mere abrasions. In all cases, however, each break in the skin was surrounded and quite well sealed by dried, sticky exudations. In a few instances the sap flowed from 1 to 2 inches down the side of the fruit from the puncture.

Before bunches of bananas are cut in the field they are stamped by the official marker of the shipper. Ten bunches stamped on June 21 were allowed to remain growing to determine whether the development that takes place during a 10-day period after the fruit is sufficiently mature for shipment lessens the general immunity it enjoys if cut when marked. It should be stated here that unless bananas are cut for shipment on the steamer for which they are marked they become too mature or, to use trade terms, too "full" or "fat," to stand without decay the 9- to 14-days' interval before they are exposed for sale in the San Francisco market. Only 9 fruits out of 505 on 4 of these 10 bunches caged with fruit flies between June 21 and June 23 bore evidences of attack, there being such evidence in 14 places. All punctures were empty, except one containing 5 eggs. These eggs had been laid in a crack caused by the decay of the blossom end of the fruit. While these eggs hatched, the larvæ immediately died. Out of 238 fruits on 2 bunches caged with fruit flies between June 23 and 26, 42 showed 159 breaks in the peel made by flies. Of these only 3 contained eggs—3, 4, and 6, respectively. An examination of these eggs on July 7 showed that while they had hatched, the larvæ were not able to mature and had died in the punctures. There were 126 attempts at oviposition in 46 out of 202 fruits on 2 bunches caged with fruit flies between June 26 and June 28; of these punctures only 2 contained eggs—1 and 3, respectively. While 3 of these eggs hatched, the larvæ died without entering the pulp. No eggs were found in 26 punctures in the peel of 15 out of 200 fruits on the last 2 bunches of those marked "June 21," and caged with fruit flies between June 28 and June 30. Plate LXI, figure 2, is reproduced from a photograph of the blossom end of a Chinese banana taken 16 days after it was

marked for shipment. The 18 punctures found on this fruit were made between June 28 and 30, or 7 to 9 days after the fruit was marked for shipment. All of these punctures were empty, and only 2 were sufficiently deep to contain eggs. The dried exudations have been removed.

Having failed to force Mediterranean fruit flies to oviposit successfully in the field in bananas sufficiently mature for the export trade, freshly laid eggs were removed from apples and placed in incisions made in the peel of bananas marked for shipment but still attached to the tree. Small cuts varying from one-fourth to one-half inch in length, extending with the grain of the peel but not quite reaching the pulp, were made. From these cuts the sap flowed so freely that it was difficult to insert eggs quickly enough to prevent them from being washed away. A total of 470 eggs inserted were sealed within the incisions with gummed labels and a thin layer of paraffin. Upon the examination of 270 eggs 2 days later, it was found that 60 eggs had hatched and that the newly hatched larvæ were alive and active within the incisions. Later examinations showed that all larvæ died without entering the pulp, even where the peel had split and exposed the latter. An examination of the 200 other eggs 9 days after they were placed within the incisions showed that 135 had hatched, but all the larvæ had died without infesting the pulp. The 275 of the 470 eggs that failed to hatch turned black. Of 65 eggs of the same lot held as a check, 57 hatched.

#### EXPERIMENTS IN THE LABORATORY

All experiments carried on in the laboratory necessarily were with fruits cut from the tree. The results were therefore obtained under conditions less normal than those obtained in the field. No experiments can be said to be carried on under field conditions unless the fruit is still growing, for as soon as it is cut its protecting sap begins to disappear.

One bunch of 55 fruits which had been cut for shipment for 24 hours was confined for 48 hours with about 500 fruit flies. An examination of the individual fruits after the bunch was removed from the cage showed 22 with a total of 28 punctures. These punctures were not opened, but the fruits were placed in jars. No adult fruit flies developed.

One bunch of 93 fruits, which had been cut for shipment for about 6 hours, was confined for 24 hours with about 300 fruit flies. On removal from the cage it was found that only 15 fruits were free from attempts at oviposition. In the remaining 78 fruits there were 342 punctures. Eggs were laid in only 7 of these 342 punctures. All eggs, or newly-hatched larvæ, died in 5 of the 7 punctures and only 3 adult flies succeeded in developing, in but one of the two fruits the pulp of which was found infested 5 days after the fruit was removed from the cage. The fruits on this bunch were almost too mature for shipment.

Twenty fruits from a bunch cut four days previously for shipment were confined in a jar containing about 400 fruit flies. Five fruits were

removed after 24 hours; 15 fruits after 72 hours. At the end of the 72 hours, or 7 days after the fruits were cut, they were beginning to turn color. In the peel of the 5 fruits first removed 58 punctures were made; yet only 1, 3, 2, and 1 fruit flies, respectively, were reared from 4 of the fruits. In the peel of the 15 fruits removed at the end of 72 hours there were 148 punctures, of which 28 contained eggs. Two days after the fruit was removed from the jars, the 28 punctures were found to contain 59 hatched eggs and 27 dead eggs. While punctures were found to be entirely empty in only 2 of the 15 fruits, adult fruit flies failed to mature in 7. There issued from the remaining 8 fruits an average of 2.2 flies, 8 being the largest number to emerge from a single fruit. Two fruits, found to contain 18 and 19 eggs, respectively, failed to produce adults.

Three fruits of the wild Borabora banana, which had been cut from the tree for two days and were still hard and yielding small quantities of sap when cut from the bunches, were placed with about 200 fruit flies for 24 hours. After removal from the cage, one fruit contained 56 eggs in its peel. The two other fruits were placed in rearing jars and produced 104 and 187 adult fruit flies, respectively. The pulp of the Borabora banana is very firm and does not decay as rapidly as does that of the Chinese or Bluefield banana.

Only 35 adults matured from 880 eggs taken from apples and placed in the peel of 44 bananas that had been cut for shipment for 24 hours. Of the 44 fruits only 31 produced adult fruit flies. Out of 107 newly hatched larvæ from apples, placed in the pulp of ripe bananas, but 33 succeeded in reaching the adult stage. Out of 137 newly hatched larvæ placed in the pulp of green bananas ready for shipment, but 40 completed the life cycle. Of these 137 larvæ 15, 52, 60, 26, and 10 were placed in bananas that had been cut from the tree 1, 2, 3, 4, and 9 days, respectively; the adults reared in the same order numbered 3, 12, 13, 5, and 7.

#### CAUSES OF IMMUNITY OF GREEN BANANAS TO FRUIT-FLY ATTACK

While it is difficult to understand why Mediterranean fruit flies have not been reared from ripe and split fruits collected on the plantations, it is not so difficult to find reasons for the immunity of fruits until they are about to turn yellow. Chemical analysis of the banana during its development, made by Mr. A. R. Thompson, of the Hawaii Agricultural Experiment Station, have shown that there exists much tannin in the peel and about the sections of which the banana fruit is composed. This tannic acid is very abundant in the green fruit, but decreases greatly in amount as the fruit becomes edible. During development, even up to the time when bananas are cut for shipment, which usually is about 12 to 16 days before they would become ripe enough to eat if kept under Hawaiian weather conditions, the peel of the fruit is so surcharged with sap laden with tannic acid that the slightest scratch of the peel produces

a flow of this staining fluid. Data on file show that practically all punctures made by female fruit flies in host fruits, the epidermis of which does not emit fluid detrimental to the pest from one or several standpoints, contain eggs, but no punctures or eggs have ever been found by the writers in the peel of bananas growing under normal field conditions and suitable for the export trade. This is true in spite of the fact that many thousand fruits have been examined.

One of the most severe tests to which any fruit can be subjected to determine whether it can support the fruit fly is to confine it closely with several hundred fruit flies of both sexes. Yet even under this extreme and unnatural condition only 1 egg was laid in 1,449 bananas exposed while still attached to the tree, and that was killed, presumably by the tannic acid in the peel. While 22 eggs were deposited in 1,145 more mature fruits, also attached to the tree, some of which were too mature for export trade, these eggs, or the larvæ hatching from them, died within the peel. When one realizes that many thousand eggs have been secured by the writers under like conditions in preferred hosts, it is clear that adult fruit flies find it extremely difficult to oviposit in fruits on the tree even under forced conditions, both when the fruit is sufficiently mature for shipment and for a period of at least nine days thereafter. At the end of this period it is considered too mature to stand transportation to the mainland. And inasmuch as shippers are paid by the bunch for their fruit, the banana markers in Hawaii are likely to mark bananas for cutting that are slightly greener than necessary in order to safeguard against unforeseen delays in shipment and crowded conditions on board the steamer which hasten the ripening process.

The difficulty experienced by the female Mediterranean fruit flies in ovipositing in green though mature fruit still attached to the tree is undoubtedly a mechanical one. She no sooner ruptures the epidermis in her attempt to form a cavity within which to deposit her eggs than she is literally forced away from her position by the exuding sap. It is possible that repeated attempts at oviposition, which are known to occur in other host fruits under natural conditions, may account for the 7 instances out of the 494 under forced or abnormal conditions when females were successful in depositing eggs. That the immunity enjoyed by Chinese and Bluefield bananas up to the time they are ready for shipment and for a period of at least nine days thereafter is due to the copious supply of sap is still further emphasized by the ease with which they become infested under similar forced conditions, or outdoor conditions, when the fruit has been cut for a short time. Fruit cut from the tree or from the bunch bleeds at the point where severed. The pressure of sap within is at once reduced and the amount of sap that exudes from cuts in the peel decreases until but little exudes after the fruit has been cut for several days. The data giving the results of close confinement of flies with bananas after they have been cut for shipment show that while the females have diffi-

culty in ovipositing as abundantly as they would in preferred hosts, such as the apple and peach, yet they find little difficulty in depositing a sufficient number of eggs to infest slightly a few of the fruits.

Inasmuch as not a single egg or newly hatched larva, as recorded in the data, was able to live in the tannin-laden peel of green though mature bananas still attached to the tree, while adults were frequently able to reach maturity in fruits severed from the tree, from which much of the sap had been drained or altered by chemical changes that proceed with the ripening process, it is evident that the sap is the chief cause of the immunity of bananas to the attack of *Ceratitis capitata*.

There is no danger of infestation during the interval between the time bananas are cut in the field and the time they are wrapped for shipment in the packing sheds.

It has been noted that oviposition has taken place under forced conditions within from 6 to 24 hours after the fruits have been cut from the tree, but that eggs deposited under such conditions have either died or the larvæ hatching from these have died without reaching the pulp. This leads to the question whether there is not danger of bananas becoming infested between the time when they are cut and the time when they are wrapped. The writers have never seen adult flies resting on bananas cut and stacked in the packing sheds, although they have personally seen many thousands of bunches ready for inspection during a 3-year period. Trade requirements demand that fruits be cut as late before the date of steamer sailing as possible. It therefore happens that bunches of bananas are inspected and wrapped within from 2 to 24 hours after they are cut, and this prompt wrapping removes all danger of infestation (Pl. LX, fig. 1, 2). From the fact that no infestation of growing bananas in condition for shipment has been known to occur in Hawaii, and that such infestations in cut fruits also suitable for shipment that are recorded have been obtained under forced conditions, whereas they have been found lacking under normal conditions, the writers believe that there is no possibility of infestation taking place between the time of cutting and that of wrapping.

#### OBSERVATIONS AND EXPERIMENTS OF OTHER ENTOMOLOGISTS

Kirk, of New Zealand, lists (4) the banana among fruits from Australia, condemned in New Zealand, in which the maggots of the fruit fly<sup>1</sup> had

<sup>1</sup> From the arrangement of the text of Kirk's bulletin (4), the Mediterranean fruit fly (*Ceratitis capitata*) is definitely listed as a banana pest. The bulletin is, however, a compilation taken for the most part verbatim from various articles on fruit flies appearing in the Reports of the Agricultural Department of New Zealand, or from circulars issued by the department. A person unfamiliar with the Australian situation is at a loss to know to which of several fruit-fly pests reference is made in the reports of fruits found infested by maggots at the ports of entry. Thus, in the Thirteenth Volume of the Agricultural Reports, 1905, where the list including the banana among those fruits found infested was originally published, no reference is made to either the Queensland or the Mediterranean fruit fly; it is merely stated that the fruits listed were burned because found infested with the "dreaded maggot." In the report for 1906 it is definitely stated that only the Queensland fruit fly (*Dacus tryoni*) was reared that year from a list of fruits including the banana. The biologist of Western Australia in his report (1) for the year 1898 stated that the Queensland fruit fly had been brought to Western Australia in bananas.



been found. French, of Victoria, Australia, states (3) that adults of this pest were reared from bananas (*Musa* sp.) exported from Queensland, Australia, and that on many occasions he has proved eggs to have been deposited in green bananas before shipment from Queensland to Melbourne. Both Kirk and French are aware that the Queensland fruit fly (*Dacus tryoni*) is a pest of bananas grown in Queensland and that confusion between the two fruit flies might occur if observations were made by untrained inspectors.

The only actual data, aside from those presented in this paper, giving the results of experimental work to determine the status of the banana as a host fruit of the Mediterranean fruit fly have been presented by Severin and Hartung (5, 6). This work was done in Honolulu and the results are of such value that they should be consulted by those interested. Their experiments, however, were carried on with fruits detached from the tree, and when green fruits were used no statement regarding the degree of greenness was made. In view of the fact that they reared specimens of the fruit fly from only two fruits out of "hundreds of bunches of bananas" examined on trees cut down in Honolulu during a campaign against mosquitoes, the writers seriously question the statement made by Severin in a later publication (7) that the "fruit fly was also bred from a half-ripe banana under field conditions." The fact that Severin reared numerous specimens of the decay flies, *Acritochaeta pulvinata*, *Euxesta annonae* Fab., and *Notogramma stigma* Fab., besides a number of species of Drosophilidae, is ample evidence that the trees from which the two fruits were taken had been cut sufficiently long for decay to have started in many fruits, had he not stated that one of the two fruits from which he reared adult flies was in a bruised and decaying condition and that its pulp had already turned yellow beneath the decayed area. It is general knowledge in Honolulu that such quantities of bearing banana trees were cut down during the campaign mentioned that the city garbage department was completely demoralized and that the trees with their fruit attached were stacked along the streets in certain parts of the city for over a week, thus giving fruit flies an opportunity to oviposit under, not growing or field, but abnormal conditions.

#### CONCLUSIONS

Since the Mediterranean fruit fly (*Ceratitis capitata* Wied.) has not been found infesting the Chinese banana (*Musa cavendishii*) or the Bluefield banana (*Musa* sp.) during the three years that the Federal Government has had charge of the inspection of export bananas in the Hawaiian Islands, it is evident that some reason exists for this practical immunity. This is the more apparent since adult flies of both sexes have been found present in all parts of banana plantations, and surrounding fruits known to be hosts have been heavily infested.

This immunity is shown to be due to the fact that neither the egg nor the newly hatched larva of the fruit fly can survive in the tannin-laden peel of green though mature fruit. In fact, the copious and sudden flow of sap from egg punctures made by fruit flies in unripe bananas renders the successful deposition of eggs in such fruits difficult and rare.

The fact that not 1 of 1,044 fruits of the Chinese banana ripening singly and prematurely among bunches growing in the field, and upon which, as in the case of other host fruits, one might expect gravid females to concentrate their attention for the purpose of oviposition, has been found to be infested leads to the conclusion that even ripe bananas are not desired as host fruits by adult fruit flies under Hawaiian conditions. On the other hand, the rearing of flies from the ripe and yellow fruits of the thin-skinned Popoulu variety, as well as from ripe fruits of other varieties under forced and unnatural conditions, leads to the equally acknowledged fact that ripe bananas in the field may serve as hosts and should therefore be properly guarded against in all quarantine work.

From the facts stated the writers believe that bunches of any variety of banana now growing in the Hawaiian Islands, when properly inspected for the removal of prematurely ripe, cracked, or partially decayed fruits, offer no danger as carriers of the Mediterranean fruit fly, provided they are wrapped and shipped in accordance with the demands of the trade and the Federal regulations.

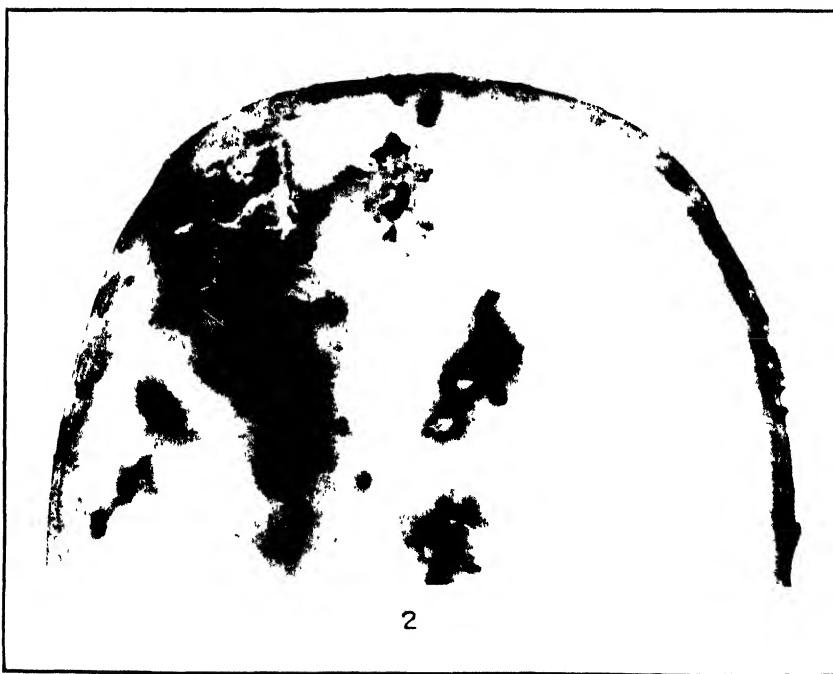
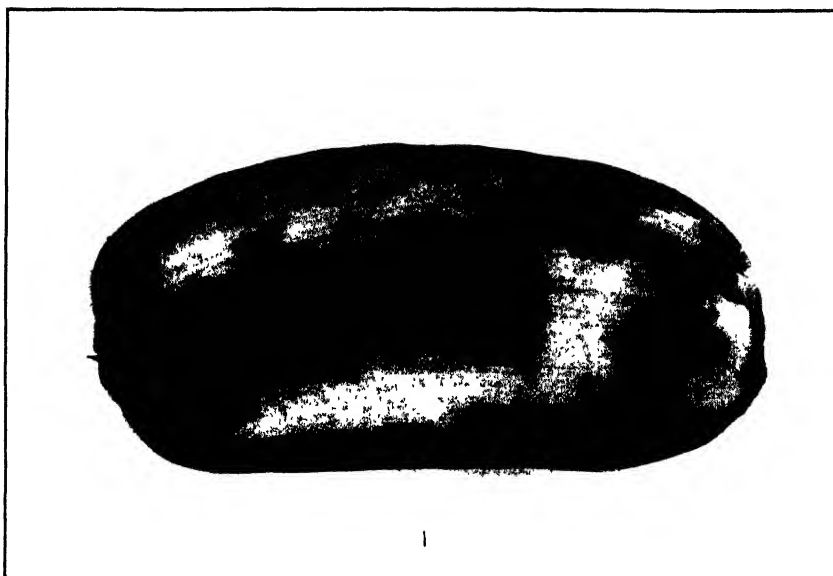
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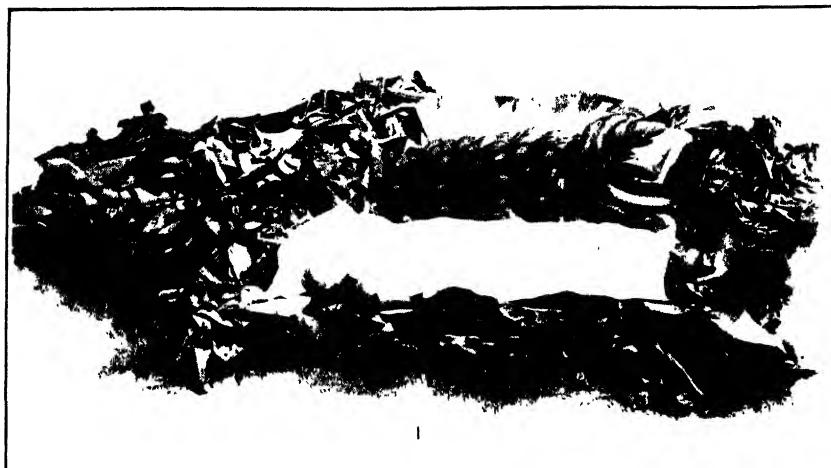
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## PLATE LIX

Fig. 1.—Popoulu variety of cooking banana found infested with the Mediterranean fruit fly. Note holes made in peel by the emerging larvæ. This fruit was fully ripe when found infested; mature fruits still green in color, present on the same bunch, were not infested.

Fig. 2.—Cross section of the Moa variety of cooking banana, showing pulp infested by larvæ of the Mediterranean fruit fly. Larvæ were found infesting the pulp of this variety only when the fruits had become mature, though not yellow in color, and when the peel had cracked sufficiently to expose the pulp, thus removing Nature's barrier to infestation.





## PLATE LX

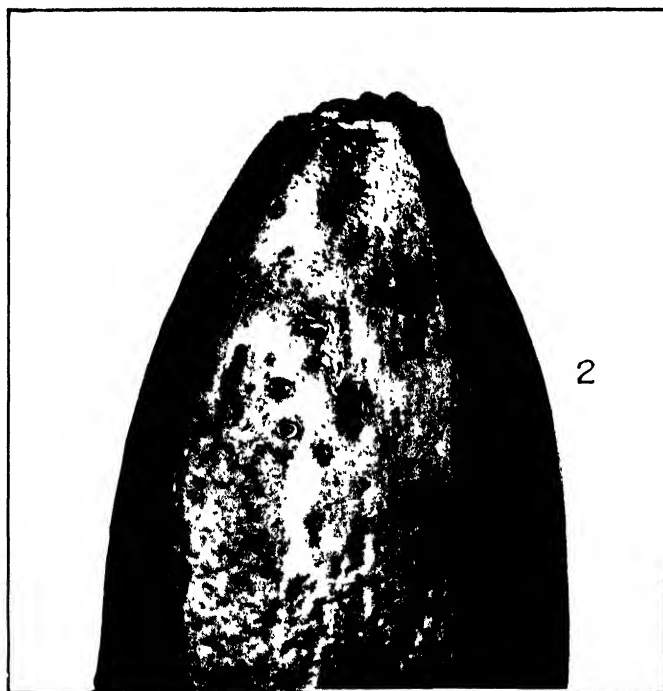
Fig. 1.—A bunch of Chinese bananas (*Musa cavendishii*). The fruit of this variety is so tender that it has to be protected during shipment by wrapping. The bunch is first wrapped in paper or cheesecloth and then in dried banana leaves, rice straw, or a mixture of the two.

Fig. 2.—A bunch of Chinese bananas wrapped in banana leaves and ready for shipment to California. Packing materials are stored for several months before use and are constantly under the supervision of inspectors to make sure that they are kept free from fruit-fly contamination.

#### PLATE LXI

Fig. 1.—Cleaning bananas in Hawaii before shipment. Every bunch of bananas shipped from the plantations in Hawaii is carefully cleaned by the Chinese growers before being inspected for the presence of ripe, cracked, bruised, or decayed fruits.

Fig. 2.—Tip of Chinese banana (*Musa cavendishii*), showing punctures made by the female Mediterranean fruit fly in attempts to deposit eggs within the peel. Though made under forced and abnormal conditions, while the fruit was still attached to the tree, and seven to nine days after it had become sufficiently mature for shipment, the 18 punctures were empty and but 2 were deep enough to contain eggs.







## PLATE LXII

Fig. 1.—Rearing cage erected over 20 Chinese banana trees and inclosing 14 bunches in various stages of development. Although adults of the Mediterranean fruit fly were introduced from time to time, none of the fruits were found infested when they became ripe.

Fig. 2.—Interior of rearing cage shown in figure 1.



# EFFECT OF CONTROLLABLE VARIABLES UPON THE PENETRATION TEST FOR ASPHALTS AND ASPHALT CEMENTS

By PRÉVOST HUBBARD, *Chemical Engineer*, and F. P. PRITCHARD, *Assistant Chemist*,  
*Office of Public Roads and Rural Engineering*

## INTRODUCTION

No one test for asphalts and asphalt cements is probably better known or more generally used than the penetration test. Many instruments have been devised for determining the consistency of these materials, but none have been generally adopted that do not substantially conform to the fundamental principles of the apparatus known as the Dow penetration machine.<sup>1</sup> This machine and others designed to give practically equivalent results are too well known to require description in this paper. In general, however, it may be said that by their use the consistency of asphalts or asphalt cements is expressed as the depth in hundredths of a centimeter that a standard needle will penetrate them vertically without external friction while the material is maintained at a stated temperature and the needle is operated under a stated load for a stated length of time. In the Dow penetration machine external friction is practically eliminated. In other satisfactory types it is reduced to an almost negligible minimum, but when operating with those in which the needle holder slides through a guiding sleeve it is most important that both the plunger and sleeve be absolutely clean and dry, as a small amount of moisture, oil, or dirt will produce considerable friction and thus retard the penetration of the needle into the sample being tested. Certain standards of temperature, load, and time have been generally adopted, and the most widely used combination is 25° C., 100 gm., 5 seconds.

Granting that the apparatus is mechanically satisfactory and that a definite standard needle is used, the test appears to be comparatively simple. It has frequently been found, however, that different laboratories, working upon samples of the same material under supposedly identical conditions of temperature, load, and time, obtain appreciably different results. The object of this investigation has therefore been to determine what effect apparently slight differences in these conditions will produce in the results of tests and also to study the importance of other controllable variables.

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<sup>1</sup> Dow, A. W. The testing of bitumens for paving purposes. *In* Proc. Amer. Soc. Testing Materials, 6th Ann. Meeting 1903, v. 3, p. 349-368, fig. 1-6. Discussion, p. 369-373. 1903.

The materials for this work were selected with the idea of obtaining products which showed rather wide differences in physical and chemical properties. For this purpose four types of oil asphalt were selected, which, being practically all bitumen, eliminated to a large extent variations due to sampling, which might have occurred in the case of native asphalts or fluxed native asphalts carrying appreciable quantities of non-bituminous material. The types represented in the following tables are produced from (1) steam-refined California petroleum, (2) steam-refined Mexican petroleum, (3) refined blended petroleum, and (4) blown petroleum. Three grades of each type were selected, having, at 25° C., under a load of 100 gm. applied for 5 seconds, penetrations of approximately 50, 100, and 150. This made 12 samples in all, and it is believed that the results obtained by their use can consistently be interpreted to cover practically all types of asphalts and asphalt cements. The more important physical and chemical characteristics of these products are shown in Table I.

TABLE I.—*Characteristics of asphalt cements*

Test.	California.			Mexican			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Specific gravity, 25°/25° C.	1.039	1.036	1.026	1.048	1.046	1.036	1.026	1.025	1.031	0.993	0.988	0.987
Melting point (cube method).....	53° C.	46° C.	42° C.	62° C.	52° C.	46° C.	62° C.	58° C.	44° C.	114° C.	82° C.	68° C.
Penetration, 25° C., 100 gm. 5 sec. ....	47	93	133	50	90	150	62	92	157	44	91	136
Penetration, 0° C., 100 gm., 1 min. ....	3	12	18	13	26	40	22	36	39	27	47	59
Penetration, 46° C., 50 gm., 5 sec. ....	Soft	Soft	Soft	227	Soft	Soft	220	310	Soft	70	176	305
Loss, 163° C., 20 gm., 5 hrs. .	.77	.90	1.28	.09	.16	.46	.38	.87	.99	.14	.20	.20
Penetration residue, 25° C., 100 gm., 5 sec.	26	45	61	37	55	87	45	58	92	38	86	118
Bitumen-soluble (CS <sub>2</sub> ) .	99.82	99.46	99.74	99.84	99.92	99.95	99.66	99.92	99.82	99.54	99.61	99.49
Organic insoluble . . . . .	.06	.36	.20	.10	.06	.05	.21	.07	.13	.27	.21	.29
Inorganic insoluble. ....	.12	.18	.06	.06	.02	.00	.13	.01	.05	.19	.18	.22
Total. ....	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Bitumen insoluble, 86° B. nap. ....	21.4	19.9	18.0	29.8	29.5	25.7	29.1	28.9	25.7	29.5	24.8	22.7
Fixed carbon . . . . .	13.4	13.3	11.0	18.1	17.6	15.3	14.0	14.2	14.5	13.3	12.4	11.3

The first consideration which naturally presents itself is the method of preparing the sample for the test. It is apparent that in order to duplicate results upon different samples of the same material the samples shall be taken so as to represent the entire body of material sampled.

It is assumed that in all instances laboratories take representative samples. The handling of the sample, once it is taken, however, is subject to a number of conditions which are not ordinarily strictly specified. In the first place, the sample must be melted by the application of heat and, to prevent any change during the melting process, it should be heated at as low a practicable working temperature as consistent with the time required to melt it. That is, all asphalts and asphalt cements tend to harden upon being heated, due either to loss by volatilization or to so-called oxidation or reaction with atmospheric air. This tendency is increased as both the temperature and time of melting are increased. The method followed in preparing all of the samples for this investigation was as follows:

About 6 ounces of each of the 12 materials were placed in pint tin cups. The 12 cups were then placed upon a  $\frac{1}{4}$ -inch asbestos board resting directly upon a gas hot plate. The samples were stirred occasionally to expedite melting, and removed from the hot plate as soon as completely fluid. At no time were the samples heated sufficiently to produce fuming. Upon removal from the hot plate the samples were poured into 3-ounce cylindrical tin dishes, measuring 5.5 cm. in diameter, with vertical sides approximately 3.5 cm. in height. While still fluid, all air bubbles which rose to the surface were removed by means of a tiny gas flame, which was rapidly passed over the surface and which merely caused the bubbles to break without in any way injuring the sample.

As the effect of the size of the container upon the results of tests had been investigated by Reeve,<sup>1</sup> it was felt that by the use of the dish above stated no danger of influencing results from this cause need be feared. In this connection it is of interest to note that Reeve's work demonstrated that a dish of 5 cm. or more in diameter could not influence the results of tests, although appreciable variations in results were in some cases caused by dishes smaller than 2.5 cm. in diameter.

#### EFFECT OF VARIATIONS IN METHOD OF PREPARING MELTED SAMPLES FOR TESTING

Undoubtedly the most common method of preparing a melted sample for the penetration test is to allow it to cool in air at room temperature for approximately an hour, then to immerse it for an hour in water maintained at the temperature at which the test is to be made. The sample is then tested under water at this temperature. In certain cases, cooling the sample in ice water or crushed ice prior to immersing it in the constant-temperature bath has been resorted to, and the penetrations so obtained have frequently been somewhat lower than those obtained by the method first described. As great a difference as 15 points in one asphalt cement

<sup>1</sup> Reeve, C. S. Effect of diameter of bitumen holder on the penetration test. *In* Proc. Internat. Assoc. Testing Materials (5th Cong. New York 1912), v. 2, no. 11, Paper 25, 4 p. 1912.

of about 150 penetration has been noted by the authors in this connection. The theory has been advanced that the ice-water cooling produces a set in the material which is not attained by the sample if it is allowed to air-cool until it has stood for a number of days. It has been further argued that the penetration at this set represents more accurately the true consistency of the material than does the penetration determined by the method first described. In order to study this matter thoroughly, different samples of each of the 12 materials were cooled and prepared for testing in a variety of ways, careful attention being paid to the time during which the sample was subjected to a given condition. These conditions are shown in Table II.

For each test under a given set of conditions samples of materials were melted and poured at the same time. In methods 1 to 6 and 15 to 23, inclusive, the melted samples were poured into the test dishes and, after standing in air for the periods indicated, were immersed in a water bath carefully maintained at 25° C. for the time selected, prior to determining their penetration. At the expiration of this time they were tested in the water bath. In methods 7 to 10, inclusive, the melted samples were poured into test dishes which had been previously packed in ice. Here they were allowed to remain until transferred to the 25° water bath. In methods 11 to 14, inclusive, the melted samples were first poured into the test dishes and allowed to cool in air as indicated, after which they were placed in an ice-water bath for definite periods of time and then immediately transferred to the 25° water bath. In methods 24 and 25, the melted samples were poured into test dishes packed in crushed ice and kept there for 1 hour. They were then removed and allowed to remain in air for 28 days, after which they were placed in the 25° water bath just prior to testing as indicated.





Table II gives the results of three determinations for each sample under each of the conditions tried. These penetrations were all taken with the same needle at different points on the surface of the sample. Reading from left to right, the first test was made at the center, the third 1 cm. from the edge of the dish, and the second halfway between the positions of the first and third tests. For the dish measuring 5.5 cm. in diameter, the first penetration was therefore taken 2.7 cm., the second about 1.9 cm., and the third about 1 cm. from the edge of the dish.

It will be noted that the time elapsing between pouring the sample into the dish and determining its penetration varied from a total of 1 hour to over 28 days; that the immersion in the water bath directly preceding the test varied from 30 minutes to 1½ hours. Upon reviewing the results given in this table, it appears evident that, in general, for any given set of conditions preceding the immersion in the water bath, a 30-minute immersion in water gave less consistent check results than a corresponding 1-hour or 1½-hour immersion. Less difference is indicated between the 1-hour and 1½-hour immersions in water, but the balance of evidence appears to favor the latter period of time in so far as uniformity is concerned, even when negligible personal errors are taken into account. Thus, out of the 11 series of comparative tests of 1 hour and 1½ hours for all 12 materials, it will be found that in 61 cases the 1½-hour immersion gave the most consistent results; in 21 cases the most consistent results were obtained with the 1-hour immersion; and in 50 cases there is no preference so far as consistency in results was concerned.

If the average of the three tests for any sample is taken for the 1-hour air cooling and 1-hour immersion in the bath, as compared with the 30-minute air cooling and 1-½-hour immersion in the bath, it will be found that they practically coincide. The fact, however, that in the latter case there is less difference between the individual results indicates that the 1½-hour immersion should have preference.

Eliminating the 30-minute immersion in the bath before making the test, and considering only the 1-hour and 1½-hour immersions in connection with short periods of prior cooling in air, Table III will be found to illustrate the differences above described. Here, comparing methods 5 and 3, it will be seen that in seven cases the most consistent results were obtained by the 1½-hour immersion; in two cases the 1-hour immersion produced the most consistent results; and in three cases there is no preference with regard to consistency in results. So far as rapidity in making the test is concerned, therefore, if a short-period air immersion is to be adopted, it would seem that 30 minutes in the air and 1½ hours in the bath prior to testing would be the most satisfactory minimum limits to adopt.

TABLE III.—Comparison of penetration tests for short periods of air cooling and immersion in 25° C. bath, 100 gm., 5 seconds

Method No.	Conditions before test.		California.			Mexican.			Blended.			Blown.		
	In air.	In 25° C. bath.	8661	8662	8663	8648	8649	8650	8694	8695	8696	8696	8697	8698
1.	30 minutes	1 hour	49	47	95	93	134	132	52	51	51	95	93	91
2.	30 minutes	1 hour	47	46	94	93	131	130	159	51	50	49	93	90
3.	30 minutes	1 hour	47	46	94	93	131	130	159	51	50	49	93	90
4.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
5.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
6.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
7.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
8.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
9.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
10.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
11.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
12.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
13.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
14.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
15.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
16.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
17.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
18.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
19.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
20.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
21.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
22.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
23.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
24.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
25.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
26.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
27.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
28.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
29.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
30.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
31.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
32.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
33.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
34.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
35.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
36.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
37.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
38.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
39.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
40.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
41.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
42.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
43.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
44.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
45.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
46.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
47.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
48.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
49.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
50.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
51.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
52.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
53.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
54.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
55.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
56.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
57.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
58.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
59.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
60.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
61.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
62.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
63.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
64.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
65.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
66.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
67.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
68.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
69.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
70.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
71.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
72.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
73.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
74.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
75.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
76.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
77.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
78.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
79.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
80.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
81.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
82.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
83.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
84.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
85.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
86.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
87.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
88.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
89.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
90.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
91.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
92.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
93.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
94.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
95.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
96.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
97.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
98.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
99.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90
100.	30 minutes	1½ hours	47	47	93	93	133	133	150	50	50	51	94	90

This being so, the average of results given in Table II can best be considered by means of Table IV, in which are given the average penetrations obtained on all of the samples under various conditions of cooling prior to  $1\frac{1}{2}$  hours' immersion in water. A study of this table shows in every case a gradual hardening or lowering of penetration as the time in air is increased. This lowering in penetration is not very pronounced in a period of 24 hours, but it increases quite appreciably in longer periods. Allowing for slight experimental errors, no difference is found to exist between the 30-minute and 1-hour exposure in air. The most marked difference is, of course, apparent between the results of 28 days in air as compared with 30 minutes in air, and the greatest difference in actual points of penetration will in every case, for a given type of material, be found for the softest grade of that type, or, in other words, for that grade which originally showed the highest penetration. It is apparent that no permanent set occurs up to a period of 28 days, but that a gradual hardening takes place. This being so, it is of interest to compare the foregoing with the results obtained by immersion in ice water prior to immersion in the water bath for  $1\frac{1}{2}$  hours at  $25^{\circ}\text{C}$ . It will be seen, in general, that but little difference in results is obtained between the samples cooled in ice water and those cooled in air, although under certain conditions for the short periods a slightly lower penetration has been secured by this means. It is safe to say, however, that the immersion of the sample in ice water does not produce a set which is comparable to any definite set produced by prolonged standing in air. This is evident from the last series of results, in which the samples which had been immersed in ice water for an hour were allowed to stand 28 days before immersing them in the water bath, the results in each case being appreciably lower than those obtained by immersing them for 1 hour in ice water and then  $1\frac{1}{2}$  hours in the bath just prior to test. There does not therefore, appear to be any good reason for cooling the sample in ice water at any time, except, perhaps, in plant-control work, where it is desired to expedite the test somewhat, and an allowance can be made for variations from the ordinary method caused by the ice-water immersion.

TABLE IV.—Comparison of average penetrations at  $25^{\circ}\text{C}$ . after  $1\frac{1}{2}$  hours' immersion in bath, 100 gm., 5 seconds

Conditions before test.			California.			Mexican.			Blended.			Blown.		
In air.	In ice.	In air.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
.....	.....	30 min	47	93	133	50	90	150	62	92	157	44	91	136
.....	.....	1 hr	46	95	134	50	90	147	61	91	158	43	91	136
.....	.....	24 hrs	45	93	131	47	86	142	58	88	150	41	89	133
.....	.....	3 days	44	90	130	45	83	139	54	85	144	42	88	128
.....	.....	7 days	43	85	125	45	78	131	53	82	142	42	85	128
.....	.....	28 days	38	79	119	39	70	124	48	73	131	38	78	112
28 days (re-melted).	.....	30 min.	46	95	133	49	91	148	61	89	156	43	93	136
.....	.....	30 min.	47	93	131	49	90	151	61	94	157	43	91	135
.....	.....	1 hr	47	94	133	49	87	146	60	92	152	42	91	136
30 min	.....	30 min.	46	93	132	49	88	146	60	93	153	43	91	135
30 min	.....	1 hr	47	92	133	48	87	142	59	92	150	43	92	134
.....	.....	1 hr	37	79	119	38	68	124	48	74	131	37	76	111

Although all of the samples examined hardened very materially upon setting for 28 days, it is of interest to note that when these samples were remelted, allowed to cool in air for 30 minutes, immersed in the water bath at 25° C. for 1½ hours, and again tested, the penetrations, to all intents and purposes, were the same as those originally obtained by the 30-minute air cooling and 1½-hour immersion in the bath. This fact does not, however, indicate that the materials do not permanently harden with age, as Hubbard and Reeve<sup>1</sup> have shown that all types of bitumen permanently harden upon prolonged exposure.

As a result of the foregoing observations, the 30-minute air cooling and 1½-hour immersion in the bath prior to the test was adopted as the method of preparing samples prior to studying the effect of the variables, temperature, load, and time.

#### EFFECT OF VARIATIONS IN TEMPERATURE

The penetration of an asphalt cement is frequently determined and sometimes specified at three temperatures. The temperature most commonly employed and at which the consistency of the material is rated is 25° C. This is known as normal temperature, and the customary load and time factors used are 100 gm. and 5 seconds.

The penetration test is next frequently made at 0° C. with a load of 200 gm. applied for 1 minute. In some cases the test may be made with a load of 100 or 200 gm. applied for 5 seconds. For this test the sample is usually packed in finely crushed ice, which completely covers it, and the needle is brought in contact with its upper surface through a hole in the ice worked out with the finger. The needle itself, as well as the exposed surface, may, therefore, at the time of test be at a somewhat higher temperature than 0°. For this reason 4° C. has been selected by some for a low-temperature test, as it is a temperature which may be accurately maintained in the water bath.

Another temperature at which the penetration test is made is 46° C. Where possible, a load of 50 gm. is applied for 5 seconds, but in the case of materials which are very soft at this temperature the 50-gm. load is applied for 1 second.

In order to study the effect of variations in temperature upon the penetration test, a number of samples of each of the 12 asphalt cements were prepared, and after cooling in air for 30 minutes were placed for 1½ hours in the bath maintained at the test temperature. The results of these tests are given in Table V.

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<sup>1</sup> Hubbard, Prévost, and Reeve, C.S. The effect of exposure on bitumens. *In Jour. Indus. and Engin. Chem.*, v. 5, no. 1, p. 15-18, fig. 1-2. 1913.

TABLE V.—Effect of variations in temperature on penetration of asphalt cements <sup>a</sup>

Temperature.	Conditions at test.			California.			Mexican.			Blended.			Blown.		
	Load.	Time.	Bath.	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
° C.	Gm.	Seconds.													
20.....	100	5	Water.	24	47	69	29	55	87	38	61	94	32	63	93
23.....	100	5	do.	37	71	106	40	73	118	50	77	126	38	81	120
24.....	100	5	do.	40	80	115	45	81	126	53	80	136	40	84	121
24.6.....	100	5	do.	46	86	121	47	86	136	56	85	145	44	89	129
25.....	100	5	do.	46	92	132	49	91	142	60	90	156	44	91	134
26.....	100	5	do.	53	100	149	54	99	153	65	97	169	47	98	144
27.....	100	5	do.	60	120	172	58	106	174	68	105	187	50	101	153
0.....	100	5	Ice....	1	10	13	10	16	23	8	11	13	11	17	20
0.....	100	5	Brine..	1	3	4	4	7	10	6	10	11	8	15	19
4.....	100	5	Water.	2	5	8	7	14	17	11	17	17	14	24	31
0.....	200	5	Ice....	10	13	18	13	26	30	13	20	24	16	28	37
0.....	200	5	Brine..	2	6	8	8	13	16	12	18	20	19	29	35
4.....	200	5	Water.	7	11	15	12	17	25	16	25	27	22	39	49
0.....	200	60	Ice....	13	17	23	28	36	39	20	37	41	28	50	62
0.....	200	60	Brine..	3	12	18	13	26	40	22	36	39	27	47	59
4.....	200	60	Water.	15	25	35	18	38	59	30	48	73	36	70	89
44.....	50	1	do.	139	239	247	99	177	268	105	152	264	45	108	175
45.....	50	1	do.	147	263	Soft.	108	188	290	118	161	281	49	116	185
46.....	50	1	do.	180	318	Soft.	116	202	308	121	174	306	54	124	200
47.....	50	1	do.	189	Soft	Soft.	126	224	Soft.	130	190	Soft.	56	129	211
44.....	50	5	do.	294	Soft.	Soft.	195	330	Soft.	190	277	Soft.	64	155	264
45.....	50	5	do.	318	Soft.	Soft.	204	Soft.	Soft.	209	286	Soft.	67	165	285
46.....	50	5	do.	Soft.	Soft.	Soft.	227	Soft.	Soft.	220	310	Soft.	70	176	305
47.....	50	5	do.	Soft.	Soft.	Soft.	245	Soft.	Soft.	244	Soft.	Soft.	73	186	Soft.

<sup>a</sup> In this and succeeding tables it will be noted that at 25° C. under a load of 100 gm. applied for 5 seconds, sample 8950 shows a materially lower penetration than in Tables II, III, and IV. No satisfactory explanation has as yet been found for this variation, as the maximum difference of eight points is too large to be attributed to experimental error. Numerous checks have been made upon the later results, which were obtained about three months after the first determinations. It is possible that the material had undergone some change during that period.

Considering first those tests made with a 100-gm. load applied for 5 seconds at temperatures ranging from 20° to 27° C., it will be seen that a difference of 1 degree makes a very decided difference in the recorded penetrations. In fact, the difference in penetration for all but the blown products and the harder grades of the other types is quite marked between 24.6° and 25°. Allowing for experimental errors, this difference of 0.4° is, in the case of sample 8963, responsible for a difference of 10 points' penetration. In general, the softer the material the greater the difference for any type. As specifications for the penetration at 25° of asphalt cements are frequently limited to a variation of 10 points, it is at once apparent that the temperature of the bath should be carefully maintained at the exact temperature required, and that accurately calibrated thermometers, which may be read to tenths of a degree centigrade, be used for this purpose.

Considering any or all of the three sets of tests made at low temperatures it is evident that the ice method is inaccurate, inasmuch as it frequently gives a higher penetration than the corresponding result with the 4° bath. It is evident, therefore, that if the temperature of 0° is used, a brine bath which may be maintained at 0° should be employed. It is further of interest to note that marked differences in penetration for all of the types are obtained between the 0° brine test and the 4° water test. From this it is apparent that the 4° test should not, as has sometimes been done, be considered the practical equivalent of a 0° test.

With regard to penetration tests at relatively high temperatures, it is of interest to note the accentuated effect of slight variations in temperature for any given material. This is due to the fact that all of the materials are much softer at this temperature. Thus, for a 50-gm. load applied for 5 seconds a difference of 24 points' penetration for 1° C. (between 45° and 46° C.) is noted for sample 8995, while for a 100-gm. load applied for 5 seconds at 25° C. a maximum difference of 9 points' penetration for 1° (between 25° and 26° C.) is shown for the same material.

#### THE EFFECT OF VARIATIONS IN LOAD

The penetration of asphalt cements is most frequently determined under a load of 100 gm. Penetration machines are, however, designed so that the combined weight of needle and plunger is 50 gm. The 100-gm. load is then obtained by placing an additional 50-gm. weight upon the plunger. A 100-gm. weight may also be used with the machine, so that loads of 50, 100, 150, and 200 gm. are possible. All of these loads are occasionally used in making the penetration test. It is clear that any variation in weight due to carelessness in manufacture or to changes brought about by the replacement of the original needle will most seriously affect the smaller loads—that is, a difference of 1 gm. should produce proportionately a more marked effect where the 50-gm. load is employed than with heavier loads. A variation of 1 gm. is, of course, much larger than would ordinarily be expected to exist in different instruments, but as great a variation as this has been noted by the writers. In order to determine the effect of variation in load, penetration tests were made upon all of the 12 samples with 1-gm. variations from the 50- and 100-gm. loads, and in addition to this the penetrations at intermediate loads between 50 and 200 gm. were determined in order to ascertain just what effect would be produced in the penetration of different types of asphalt cements by changes in load when the penetrations were all made for 5 seconds at a temperature of 25° C. The results of these tests are given in Table VI.

TABLE VI.—*Effect of variations in load on penetration of asphalt cements, 25° C., 5 seconds*

Load.	California.			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
Gm.												
49.....	31	59	89	32	60	96	40	60	106	26	53	83
50.....	32	60	90	32	61	97	40	60	106	26	54	83
51.....	32	61	91	33	62	98	40	60	106	26	54	83
60.....	35	67	98	36	67	110	43	63	120	29	63	94
75.....	40	76	112	41	77	123	48	75	133	36	72	111
90.....	43	85	123	45	85	134	54	84	147	39	82	124
99.....	46	91	132	48	90	140	60	90	155	41	89	134
100.....	46	92	132	49	90	142	60	90	153	42	90	134
101.....	46	92	132	49	91	142	60	90	156	43	90	135
125.....	51	101	146	54	101	159	65	101	173	51	105	157
150.....	59	113	160	60	113	178	76	113	192	58	118	182
200.....	68	134	178	72	129	211	90	134	218	72	153	231

Upon reviewing these results it will be noted that a variation of 1 gm. in no case produces an appreciable variation in results. In fact, the greatest variation is found to be one point penetration, and, in many cases, no difference in penetration is to be observed. It is therefore obvious that errors due to the calibration of the weights are practically negligible.

In connection with the series of tests for any individual material, it is of interest to note that within certain limits the increase in penetration is almost proportional to the increase in load. In other words, practically a straight-line curve may be obtained by plotting for any material the load against the corresponding penetration and connecting these points. If this is done the projection of the line to the axis representing increments of load will not hit this axis at its intersection with the axis representing increments of penetration. In general, it appears that blown asphalts possess less surface tension and adhesiveness than steam-distilled asphalts. The penetration of a blown asphalt therefore represents more nearly the actual distance which the needle enters the sample. In the case of steam-distilled asphalts the surface of the sample is markedly depressed by the needle, and probably proportionally greater retardation of its movement is produced by material which adheres to it.

It is of interest to note that a steam-distilled asphalt having a higher penetration than a blown asphalt at 25° C. under a load of 50 gm. applied for 5 seconds may have a lower penetration than the same blown asphalt at 25° under a load of 100 gm. applied for 5 seconds. For this reason the relative penetrations of different types of asphalt do not necessarily indicate their relative hardness.

As would naturally be supposed, in general, the greatest variations in penetrations due to variations in load are obtained upon the softer materials or those showing the highest penetration at any given load. The blown products, however, show more variation than do the other types. This is probably due to the fact that the effect of surface tension and adhesion is less pronounced with the blown products than with the steam-distilled products.

It was thought unnecessary to study the effect of variations in load at other temperatures and for other periods of time, as there was no reason to suppose that the results would be different in character from those given. The changes in time and temperature would merely change the penetration of the material and should give results comparable with those obtained upon softer or harder grades of the same type.

#### EFFECT OF VARIATIONS IN TIME

Penetration determinations are ordinarily made for a period of 5 seconds, especially where the 100-gm. load is employed. In the case of materials which are quite hard they may be made for a period of 1 min-

ute and usually under a load of 200 gm. This is done in most 0° or 4° C. tests. If a material is normally very soft or becomes very soft at 46° a 1-second test under a load of 50 gm. may be used. The time of test may be controlled by means of a swinging pendulum, a second clock, or metronome. The last is to be preferred because it leaves the eye free to watch the test itself and at the same time incurs less chance of error.

In order to determine the effect of variations in time upon the penetration test, samples of all 12 asphalt cements were prepared and tested at 25° C. under a load of 100 gm. applied for periods ranging from 1 to 10 seconds. The results of these tests are given in Table VII, in which every value recorded represents an average of a number of determinations made directly for the intervals of time stated.

TABLE VII.—*Effect of variations in time on penetration of asphalt cements, 25° C., 100 gm.*

Time.	California			Mexican.			Blended.			Blown.		
	8961	8962	8963	8948	8949	8950	8994	8995	8996	8956	8957	8958
<i>Seconds.</i>												
1.....	25	50	62	26	48	77	35	53	80	33	63	91
2.....	32	63	84	33	62	96	45	67	105	36	73	108
3.....	38	74	104	37	73	116	52	76	124	39	80	117
4.....	42	84	118	44	81	132	56	84	136	42	85	126
4½.....	45	88	126	45	84	140	58	88	148	43	88	131
5.....	47	93	132	48	89	145	60	91	155	44	90	135
5½.....	49	98	137	50	91	150	62	94	160	45	91	138
10.....	64	130	183	61	116	192	74	115	210	49	104	159

Upon reviewing these results it will be noted that for any material a greater number of points penetration is recorded for the first second than for any other one second. In general, upon the basis of a 5-second test it will be found that about 50 per cent of the penetration occurs during the first second for all but the blown type. With this type, owing probably to less surface tension and adhesion, considerably more than 50 per cent of the total 5-second penetration occurs during the first second. After the first second there is a decided tendency for the penetration to become less and less for each succeeding second. But with the softer grades of material a difference of one-half second from the 5-second test may make as much as 7 points difference in penetration. It is evident, therefore, that for accurate work in the 5-second test the time of penetration should be controlled to within less than half-second variations. From numerous tests it appears that if a metronome is used, the time of penetration may be controlled by any careful operator to within a maximum variation of one-fifth second from the selected time of test, and this is believed to be sufficient for all practical purposes.



## SUMMARY AND CONCLUSIONS

For the sake of convenience, the more important conclusions regarding the method of making penetration tests, which have been reached as a result of this investigation, are summarized below.

(1) Melted samples should be cooled for not less than 2 hours prior to test, and should be tested upon the same day that they are melted, preferably after 2 or 3 hours.

(2) Samples should be maintained at the testing temperature for not less than 1 hour, and preferably for  $1\frac{1}{2}$  hours prior to test.

(3) Upon standing in the air, prepared samples show a decreasing penetration, but no definite end point or set is produced up to 28 days.

(4) In ordinary laboratory work there is no apparent advantage in cooling samples in ice or ice water prior to determining their penetration at higher temperatures. Cooling in ice water is therefore not recommended.

(5) Samples should be maintained and tested within  $0.1^{\circ}\text{C.}$  of the desired temperature for accurate work, as a variation in temperature of less than  $0.5^{\circ}$  in temperature may produce a decided difference in results.

(6) Tests at  $4^{\circ}$  are not the practical equivalent of properly made tests at  $0^{\circ}$ .

(7) When making tests at  $0^{\circ}$ , samples should not be packed in crushed ice, but should be immersed in a brine bath.

(8) The increase in penetration of a material determined under given conditions of temperature and time is, within certain limits, almost proportional to the increase in load. For the 100- and 200-gm. loads variations of as much as 1 gm. do not as a rule seriously affect determinations. It is, however, recommended that in all cases the load should not vary more than 0.2 gm. from that desired.

(9) In any test, proportionally the greatest number of points penetration is obtained during the first second. In the 5-second test approximately one-half of the total penetration is obtained during the first second. A variation of one-half second may, however, produce an appreciable variation in results.

(10) A carefully calibrated metronome is recommended for securing the proper time control.

(11) Aside from possible variations in needles, it is believed that variations in results obtained upon the same material by different laboratories are more probably due to unobserved variations in the methods of preparing the sample and to the control of temperature than to any other causes.

(12) It is believed that a study of the penetration of various types and grades of bituminous materials under a variety of conditions of temperature, load, and time may throw considerable light upon their other physical and chemical characteristics, and may serve as a possible means of identifying their origin and method of manufacture. The writers propose to continue work along this line.

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### EFFECTS OF REFRIGERATION UPON THE LARVÆ OF *TRICHINELLA SPIRALIS*

By B. H. RANSOM,

*Chief, Zoological Division, Bureau of Animal Industry*

#### INTRODUCTION

Prior to recent investigations, the first of which were briefly reported in a short article which appeared about two years ago (Ransom, 1914), it had been generally accepted as an established fact that the larvæ of *Trichinella spiralis* are very resistant to cold and that they survive exposure to temperatures much below the freezing point of water. In the article referred to, however, it was shown that former ideas concerning the resistance of trichinæ to cold were erroneous, and that as a matter of fact low temperatures have a very pronounced effect upon the vitality of these parasites. As a precise knowledge of the effects of refrigeration upon trichinæ is of considerable importance, an extended investigation has been made, the results of which are recorded in the present paper.

#### HISTORICAL SUMMARY

The following summary covers all of the published reports of experimental work on the effects of cold upon trichinæ so far as they could be traced in the literature.

Leuckart (1863a, p. 120) states that trichinæ are in the highest degree resistant to cold. He exposed some trichinous meat outdoors during cold January weather ( $-16^{\circ}$  to  $-20^{\circ}$  R.;  $-4^{\circ}$  to  $-13^{\circ}$  F.;  $-20^{\circ}$  to  $-25^{\circ}$  C.) for three days and nights. After thawing the meat, he fed it to a rabbit, which died a month later and was found to be very heavily infested with trichinæ. In another publication (1866a, p. 91) Leuckart notes that the place in which this meat was kept was somewhat protected, and it may therefore be presumed that the temperature to which the meat was actually exposed was probably not as low as indicated by the figures given. Leuckart remarks, however, that the meat was solidly frozen throughout.

Fiedler (1864, p. 466) exposed the leg of a trichinous rabbit to an outdoor temperature of  $-15^{\circ}$  to  $-17^{\circ}$  R. ( $-1.75^{\circ}$  to  $-6.25^{\circ}$  F.;  $-18.75^{\circ}$

to  $-21.25^{\circ}\text{C.}$ ) from January 6, 5 p. m., to January 7, 8 a. m.—i. e., for 15 hours. Examined on a warm stage, the trichinae showed no movement. Some of the meat was fed to two rabbits on January 7, and on February 7, a month later, the rabbits were killed. In one of them a few encysted trichinae were found. On January 16 he fed two rabbits with some trichinous meat which had been cut in fine pieces and exposed for 18 hours to a temperature of  $-11^{\circ}$  to  $-12^{\circ}\text{R.}$  ( $7.25^{\circ}$  to  $5^{\circ}\text{F.}$ ;  $-13.75^{\circ}$  to  $-15^{\circ}\text{C.}$ ). On February 14 the rabbits were killed and carefully examined. No trichinae were found.

Rupprecht (1864a) exposed trichinous meat during one night to an outdoor temperature of  $-18^{\circ}\text{R.}$  ( $-8.5^{\circ}\text{F.}$ ;  $-22.5^{\circ}\text{C.}$ ) and found that the vitality of the trichinae was not affected.

Kühn (1865b), according to Leuckart (1866a, p. 91), found that trichinous meat kept in an ice chamber for  $1\frac{1}{2}$  months was still infectious and that the trichinae had lost their vitality only after the meat had been kept for 2 months in the ice chamber, the temperature of which was not given.

Gibier and Bouley (1882a) exposed some trichinous ham for 4 hours to temperatures of  $-27^{\circ}\text{C.}$  ( $-16.6^{\circ}\text{F.}$ ) and  $-20^{\circ}\text{C.}$  ( $-4^{\circ}\text{F.}$ ). In the first case the interior temperature reached  $-20^{\circ}\text{C.}$  ( $-4^{\circ}\text{F.}$ ) and in the second  $-15^{\circ}\text{C.}$  ( $5^{\circ}\text{F.}$ ). All of the trichinae were found to be dead. They showed no movement when warmed, and they stained in a few minutes with anilin blue, methyl-anilin violet, and picrocarminate of ammonia. Some of the meat which had been frozen was fed during 8 days to five birds, which when examined later showed no trichinae in the intestine; nor had any been found in the feces. Trichinae from portions of the ham which had not been frozen were active when warmed to  $40^{\circ}\text{C.}$  and remained transparent and colorless for several days in staining solutions. Five birds of the same kind and age as those to which the frozen meat had been fed were similarly fed with the ham which had not been frozen, and large numbers of trichinae were afterwards found in the feces and intestines.

These experiments of Gibier and Bouley seemed to show pretty clearly the destructive effects of low temperatures upon trichinae, but later Gibier (1889a) came to the opinion that the death of the parasites was to be explained on the ground that they had already suffered a reduction in vitality from the action of salt, and, hence, readily succumbed to freezing. This opinion was based on the results of an experiment in which he exposed small fragments of fresh trichinous pork for 2 hours to a temperature of  $-20^{\circ}$  to  $-25^{\circ}\text{C.}$  ( $-4^{\circ}$  to  $-13^{\circ}\text{F.}$ ). The parasites, when afterwards examined on a warm stage, were found to have lost none of their activity.

From the foregoing it would appear that the usual statements found in articles relating to *Trichinella spiralis* as to the resistance of this parasite to low temperatures have their principal basis in Leuckart's

single experiment, to which may be added, as supplementary support, Fiedler's first experiment, Rupprecht's experiment, and Gibier's experiment, a total of four experiments. Kühn's experiment perhaps has been considered as affording additional supporting evidence. The results of Fiedler's second experiment do not offset the results of his other experiment, nor those of Leuckart's and Gibier's experiments, as the failure to get an infestation in the two rabbits which were fed meat exposed for 18 hours to a temperature of  $7.25^{\circ}$  to  $5^{\circ}$  F. might have been brought about by something else than low temperature. Likewise, the results of Gibier and Bouley, when compared with those of Leuckart, Fiedler, and Gibier, tend to show only that trichinae are sometimes killed when exposed for a short time to temperatures below zero. The later explanation by Gibier (1889) that the trichinae used in these experiments had lost so much vitality on account of previous salting of the meat that they succumbed, whereas they would not have done so if the meat had been fresh, has been accepted by those authors who have mentioned Gibier and Bouley's work. It should be noted, however, that in the experiment upon which Gibier (1889) based his explanation of the results of the earlier experiments by himself and Bouley the meat was exposed for only 2 hours as compared with 4 hours in the earlier experiments.

So far as appears in the available literature, after the later experiments conducted by Gibier (1889), no further work on the effects of cold upon trichinae was done until the investigations undertaken by the present writer, 25 years later, the first of which were recorded briefly in an article (Ransom, 1914) already mentioned.

A few additional data gathered in these investigations were given in a later paper (Ransom, 1915).

Recently Schmidt, Ponomarer, and Savelier (1915) have published a preliminary report of some investigations of the effects of cold upon trichinae in which they state that a long series of experiments has led to the following results:

1. A temperature of  $0^{\circ}$  C. ( $32^{\circ}$  F.) has no influence upon the vitality of encysted trichinae, even though it acts during a period of 11 days.
2. A temperature of  $-6^{\circ}$  C. ( $21.2^{\circ}$  F.) is easily withstood by trichinae during a period of 10 days, but they revive slowly.
3. A temperature of  $-9^{\circ}$  C. ( $15.8^{\circ}$  F.) is sometimes fatal, but not always. The results are not always the same; they are uncertain.
4. A temperature of  $-15$  to  $-16^{\circ}$  C. ( $5^{\circ}$  to  $3.2^{\circ}$  F.) is always fatal; the trichinae never revive.

Winn (1915) exposed some trichinous meat out of doors away from the sun in February, 1914, for 16 days, at an average mean temperature of  $-18.8^{\circ}$  C. ( $-2^{\circ}$  F.) with a minimum of  $-25^{\circ}$  C. ( $-13^{\circ}$  F.) and a maximum of  $-12.2^{\circ}$  C. ( $10^{\circ}$  F.). Nine guinea pigs were fed upon this meat, and none became infested.

## EXPERIMENTAL WORK

## DESCRIPTION OF EXPERIMENTS

The first experiment was carried out in Chicago in September, 1913. The carcass of a naturally infested trichinous rat killed on September 11 was inclosed in a tin can and kept in a refrigerator until September 16, when it was placed in a refrigerated compartment known as a "freezer" in one of the meat-packing establishments, where it remained for nearly 6 days—i. e., 5 days, 22 hours. During this time the temperature (as recorded by a thermometer not compared with a standardized thermometer), read once daily, varied from  $-3^{\circ}$  to  $-10^{\circ}$  F.<sup>1</sup> When removed, the rat carcass was allowed to thaw by exposure to ordinary room temperature, after which eight trichinae were isolated by dissection. Examined in water on a warm stage, they were found to be shrunken and motionless. They were left in a moist chamber and again examined the following day, when they were found to be no longer shrunken, but exhibited no movement. Two more trichinae, isolated from the meat the day after removal from the freezer, were also found to be inactive. A guinea pig was fed some of the meat from the rat carcass on September 25 and was found to be free from trichinae when examined on October 25.

The failure to discover any evidence of life among the trichinae isolated from the frozen rat carcass led to further experiments.

In experiment 2, a small piece of the diaphragm of another trichinous rat, after the carcass had been kept in an ice box for 11 days, was sealed in a vial and kept in a freezing mixture at a temperature of  $4^{\circ}$  to  $10^{\circ}$  F. for 30 minutes. No active trichinae were found on examination after thawing. The rest of the carcass of the same rat was then inclosed in a tin can and placed in a freezer maintained at a temperature of  $13^{\circ}$  to  $15^{\circ}$  F., recorded by means of a thermometer (six readings daily), afterwards compared with a standardized thermometer (experiment 3). After nearly 2 days ( $45\frac{1}{2}$  hours) the can was removed from the freezer. Trichinae isolated by dissection soon after the meat had thawed and examined in water on a warm stage were found to be shrunken and motionless, but resumed their normal appearance and became active in 10 to 30 minutes.

In experiments 4, 5, and 6, pieces of diaphragm of an artificially infested rabbit were sealed in small vials and exposed to a temperature of  $-6^{\circ}$  F. for 10, 20, and 30 minutes, respectively; none of the trichinae isolated by dissection from the meat after thawing showed any activity, and guinea pigs fed with the meat failed to become infested.

In experiment 7 the carcass of a naturally infested rat was kept in a tin can in a freezer at  $13^{\circ}$  to  $15^{\circ}$  F. (six readings daily; thermometer

<sup>1</sup> Because of the practical bearing of the experiments upon the meat-packing industry, refrigeration temperatures are given according to the Fahrenheit scale, which is the only temperature scale in common use in the United States.

compared with a standardized thermometer) for a period of nearly five days. *Trichinae* isolated by dissection showed slight activity on a warm stage.

The methods employed in experiments 8 to 127 and a general discussion of these experiments are given in the following pages, but it has been found expedient in order to save space to omit from the narrative statements of the results. These are later set forth in tabular form (Tables I, II).

In experiment 8, a leg of the rabbit referred to in experiments 4 to 6 was inclosed in a tin can and kept in a freezer at  $-2^{\circ}$  F. for  $43\frac{1}{2}$  hours (thermometer not compared with a standardized thermometer; one reading daily). The next day after its removal from the freezer some of the meat was chopped in fine pieces and placed in the incubator ( $38^{\circ}$  to  $40^{\circ}$  C.) in a beaker containing an artificial gastric juice (water; hydrochloric acid, about 0.35 per cent; and pepsin—exact quantity of pepsin used not recorded). Unfrozen meat from the same rabbit was similarly treated, using a portion of the same lot of digesting fluid. After incubating overnight, the sediment in the beakers was washed with several changes of water by decanting and settling. *Trichinae* from the two lots of digested meat were then examined in water on a warm stage and the number of active and inactive individuals recorded. A guinea pig was fed some of the meat after it had thawed, and another guinea pig was fed some unfrozen meat from the same rabbit as a control, both being killed and examined for trichinae after the lapse of a month.

Substantially the same methods of examination and feeding of test animals, with control examinations and feedings, were employed in experiments 9 to 22b. Meat from trichinous rats and rabbits was inclosed in tin cans, placed in freezers, which were maintained at various temperatures, and kept there for various periods. Portions of the meat were digested in artificial gastric juice and washed and examined as in experiment 8. Guinea pigs were used as test animals in experiments 9 to 15, white rats in experiments 16 to 22b.

In experiments 23 to 34 the carcass of a hog which had been artificially infested with trichinae by feeding trichinous meat from various sources at intervals during a period of four months was hung in a freezer, the temperature of which was recorded by means of a thermometer (six readings daily) which had been compared with a standardized thermometer. The dressed carcass weighed about 150 pounds. The head was removed and kept unfrozen in a cooler to provide material for control examinations and feedings. From time to time portions of the carcass were removed for examination and test feedings. The same methods of examination were followed as in experiment 8. Test animals, usually white or hooded rats, were fed, and one lot of rats was fed unfrozen meat from the same carcass as a control.

In experiments 35 to 48 the carcass of another hog artificially infested as in the case of the hog used in experiments 23 to 34, weighing about 125 pounds dressed, was split in halves, which were hung in two freezers kept at different temperatures. The same procedure as to examination and feeding of test animals was followed as in experiments 23 to 34.

In experiments 49 and 50 digested meat from a trichinous rabbit, after washing and sedimenting with water, was inclosed in small vials, frozen by immersion in a freezing mixture, and the trichinæ, after thawing, examined on a warm stage.

In experiments 51 to 55, a hog artificially infested as in experiments 23 to 48 was slaughtered, and meat from the carcass inclosed in five 1-pound cans which were placed in the center of five barrels 28 inches high by 17 inches in diameter at the ends and 20 inches in diameter at the middle, each containing about 250 pounds of pork trimmings. The head of the carcass was kept unfrozen in a cooler to provide material for control examinations and feedings. The barrels were placed in a freezer the temperature of which was recorded six times daily by means of a thermometer which had been compared with a standardized thermometer. The barrels were removed from the freezer after 7, 8, 9, 10, and 11 days, respectively, and allowed to thaw sufficiently to permit the removal of the cans of trichinous meat. Examinations of the meat were made as in experiment 8. White or hooded rats in lots of five or six were fed some of the meat on several successive days, a separate lot being fed from each can.

In connection with experiments 51 to 55, it may be noted that in another experiment it was found that the interior temperature (determined by an electrical thermometer) of a barrel containing 250 pounds of pork trimmings did not fall to the temperature of the freezer (5° to 7° F.) from an initial temperature of 32° until the barrel had been in the freezer for eight days.

In experiments 56 to 64 the carcass of the hog from which meat was taken for use in experiments 51 to 55 was hung in the same freezer, and portions were removed from time to time for examination and feeding of test animals, following the same procedure as in those experiments.

In experiments 1 to 64, specially reared white or hooded rats were used as test animals whenever possible, but in some cases it was necessary, on account of the lack of a sufficient supply, to utilize rats whose previous history was not fully known; and in other cases the use of guinea pigs was necessary. In the remaining experiments, 65 to 127, only white or hooded rats were used which had been specially reared for the purpose on food from which there was no possibility of acquiring an accidental infection with trichinæ.

The meat from six hogs was used in experiments 65 and 65a. Four of these were artificially infested hogs which had been fed with trichinous pork several months before they were slaughtered, in October, 1914.

The two others slaughtered about the same time were naturally infested, having been found trichinous on microscopic examination. A shoulder was taken from each carcass and kept unfrozen in a cooler to provide material for control examinations and feedings.

In experiment 65, trimmings were taken from each of the six carcasses and a quantity weighing 106 pounds was inclosed in a wooden box measuring 28 by 19 by 6½ inches. The box was placed in a freezer, where it remained for 19 days, the temperature of the freezer being recorded three times daily by a thermometer which was afterwards compared with a standardized thermometer. After removal from the freezer the box was allowed to thaw for 2 days. A portion of the meat was then taken from the middle, passed twice through a meat chopper, and digested and examined as in experiment 8, a control examination being made of a mixture of unfrozen meat from the same carcasses similarly prepared and digested. A definite formula was followed in the preparation of the digesting fluid, which was mixed in the following proportions: Water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm. Five rats were fed some of the ground meat, 50 gm. of which were placed in their cage on each of three days, a total of 150 gm., an average of 30 gm. per rat. As controls five rats were fed once an average of 10 gm. of unfrozen meat from one of the hog carcasses, another lot of five, 10 gm. each from another carcass, and so on—i. e., 30 rats in all, 5 for each hog.

In experiment 65a, some of the same lot of frozen trimmings were used and were examined and fed to five rats, following the same methods as in experiment 65. In this case the trimmings had been made into sausage meat after thawing, a curing mixture having been mixed with the meat, containing salt equivalent to 3⅓ per cent of the weight of the meat. After the addition of the curing mixture and until it was prepared for artificial digestion and feeding of test animals, the meat remained for two days in a cooler at a temperature of 36° to 37° F. Analysis showed that the meat contained 3.12 per cent of salt. In preparing it for examination and feeding tests, the meat, immediately after it was ground up, was washed in water to remove the salt.

In experiment 66, 8 pounds of meat from a naturally infested hog were inclosed in a box 15¾ by 9 by 3 inches and placed in a freezer the temperature of which was recorded three times daily by means of a thermometer which was afterwards compared with a standardized thermometer. After 19 days the box was removed and some of the meat was examined and fed to test animals, following the methods used in experiment 65. As controls, five rats were fed 50 gm. of unfrozen meat from the same carcass, an average of 10 gm. per rat.

In experiments 67 to 71, meat was taken from the same carcasses as that used in experiment 65. Mixed meat from the six hogs was placed in five half-pound tin cans. Each can contained an approximately



equal quantity of meat from each hog. Two of the cans were placed in freezers, one maintained at  $-9^{\circ}$  to  $0^{\circ}$  F. (three readings daily; thermometer not compared with a standardized thermometer), the other maintained at  $10^{\circ}$  to  $12^{\circ}$  (three readings daily; thermometer compared with a standardized thermometer). When removed from the freezers, the cans were thawed at room temperature, the thawing of the meat from the can taken from the second freezer ( $10^{\circ}$  to  $12^{\circ}$ ) being hastened by pulling the pieces of meat apart (experiment 71). The examination and the feeding of test animals were carried out in the same manner as in experiment 65. The three other cans were placed in the center of boxes 28 by 19 by  $6\frac{1}{2}$  inches, each containing about 100 pounds of pork trimmings. These boxes were placed in the same two freezers as the loose cans, two in the freezer maintained at the lower temperature (experiments 67, 68), the third box in the other freezer (experiment 70). When removed from the freezer, the boxes were allowed to thaw for two days. The cans were then removed and the meat examined and fed to rats, following the methods used in experiment 65.

In experiments 72 to 76 meat was taken from an artificially infested hog which had been fed trichinous meat several months prior to its slaughter in November, 1914, and this meat was inclosed in five half-pound tin cans. A ham from the carcass was kept unfrozen, at first in a cooler and afterwards in an ice box, to provide material for control examinations and feedings. Two of the cans were placed in a freezer maintained at a temperature of  $-9^{\circ}$  to  $2^{\circ}$  F. (three readings daily; thermometer not compared with a standardized thermometer), two in a freezer maintained at a temperature of  $10^{\circ}$  to  $13^{\circ}$  (three readings daily; thermometer compared with a standardized thermometer), and the fifth in the center of a box 28 by 19 by  $6\frac{1}{2}$  inches, containing about 100 pounds of pork trimmings, this box being placed in one of the freezers ( $-9^{\circ}$  to  $2^{\circ}$ ) just mentioned.

The meat in the loose cans was allowed to thaw rapidly when removed from the freezers; that in the box required two days to thaw so that the can could be readily removed. The same methods of examination were followed as in experiment 65, except that some of the examinations were made in a 0.6 per cent salt (sodium chlorid) solution following digestion of the meat, the digested meat in those cases being washed with a 0.6 per cent salt solution instead of water. The use of a 0.6 per cent salt solution was adopted when it was discovered that trichinæ digested out of meat commonly become inactive if kept from a half an hour to several hours in water at a temperature of  $32^{\circ}$  to  $40^{\circ}$  C. This does not occur in cold water nor in warm salt solution. In the earlier experiments the use of plain water probably led to no misleading results, however, as every examination was controlled by an examination of unfrozen meat similarly treated. The same methods with reference to the feeding of test animals were followed in experiments 72 to 76 as

in No. 65. Four rats as controls were fed a total of 20 gm. of meat on July 8, 1915, from the ham which had been kept unfrozen since the slaughter of the hog—nearly eight months. No infections resulted. The trichinæ had evidently died. Examination on August 25 of some of the meat after artificial digestion showed only a few trichinæ. These were dead and disintegrated. There is little doubt, however, that if control animals had been fed early enough, they would have become infested, since trichinæ from the unfrozen meat examined after artificial digestion as late as three weeks after slaughter of the hog were quite lively and appeared altogether normal.

In experiments 77 to 87, meat from five trichinous hogs was used. Three 1-pound cans ( $5\frac{1}{2}$  by  $2\frac{3}{4}$  inches) were filled with meat from the first hog. One of the cans was placed in the center of a box 28 by 19 by  $6\frac{1}{2}$  inches, containing about 100 pounds of pork trimmings, and another in the center of a barrel of pork trimmings weighing 383 pounds net (dimensions of the barrel not recorded). Two cans were filled with meat from the second hog and two each in the case of the third, fourth, and fifth hogs, and one can of meat from each hog was placed in the center of a box of trimmings, as was done with one of the cans of meat from the first hog. A shoulder from each hog was kept unfrozen to provide material for control examinations. These shoulders were kept in a cooler or an ice box, except during the time when they were in transit between Chicago and the Washington laboratory.

The five boxes and the barrel were placed in a refrigerated compartment or freezer, maintained at a temperature of  $-2^{\circ}$  to  $5^{\circ}$  F. The five loose cans were placed in a freezer maintained at  $12^{\circ}$  to  $16^{\circ}$ . The boxes were kept in the freezer for 15 days, the barrel for 23 days, and the loose cans for 17 days. During the time the meat was in the freezers the temperature was recorded three times daily, using a thermometer which was afterwards compared with a standardized thermometer, and found to be substantially correct. The temperature of the freezer in which the boxes and the barrel were kept varied from  $-2^{\circ}$  to  $5^{\circ}$  during the time the box and barrel containing meat from the first hog were in it. During the time the four other boxes were in this freezer the temperature varied from  $-2^{\circ}$  to  $2^{\circ}$ . The temperature of the freezer in which the five loose cans were kept varied between  $12^{\circ}$  and  $16^{\circ}$  during the time the can of meat from the first hog was in it, and between  $13^{\circ}$  and  $15^{\circ}$  during the time the four other cans were in it.

When the boxes were removed from the freezers after 15 days' exposure to cold, they were allowed to thaw slowly until the cans could be removed, which required two days (three days in one case, experiment 77). The thawing of the barrel required five days. After removal the cans were forwarded by mail from Chicago to Washington, where they were kept after arrival in an ice box or in a cooler (temperature, above  $32^{\circ}$  F.) until they could be examined. The time elapsing between removal from the

freezer and the placing of the meat in artificial gastric juice in preparation for examination varied between 6 and 12 days.

In preparing the meat for examination and feeding tests, the contents of the can were passed twice through a meat chopper, thoroughly mixing the ground meat together. Fifty gm. of ground meat from each can were placed in a beaker containing 600 c. c. of a freshly prepared artificial gastric juice made by the following formula: Water 1,000 c. c., hydrochloric acid (sp. gr. 1.19) 10 c. c., scale pepsin (U. S. P.) 2.5 gm. (experiments 77, 78); or the same formula modified by the addition of 6 gm. of sodium chlorid (experiments 79 to 87). The contents of the beaker were then stirred and carefully warmed to 40° C. and the beaker placed in an incubator (37° to 40° C.) for 18 to 24 hours. After removal from the incubator the supernatant fluid was decanted off, salt solution (0.6 per cent) added, the contents of the beaker stirred, allowed to settle, again decanted, more salt solution added, and so forth, until the supernatant fluid remained clear and transparent. As a control upon a possibly injurious effect of the digestant on the trichinae, 50 gm. of ground unfrozen meat from the same carcasses as the frozen meat to be examined were placed in 600 c. c. of the same lot of digestant prepared for digesting the meat which had been frozen, put into the incubator, and removed at the same time as the other, washed in the same manner, and handled in all respects exactly the same as the meat which had been frozen. The sediment which remained in the beakers after washing and decanting was examined in salt solution (0.6 per cent) on a warm stage under the microscope.

In the tests on animals five white or hooded rats, reared from birth on food from which there was no possibility of acquiring an accidental infection with trichinae, were used for testing each lot of meat. The five rats were kept together in a cage and 50 gm. of the ground meat were placed in the cage each day for three days, a total of 150 gm. of meat, or an average of 30 gm. per rat. The cage was watched to see that the meat was all eaten. It was usually eaten promptly. The rats which died within the first two weeks were examined for the presence of trichinae in the intestine as well as in the muscles. In the case of those which died later only the diaphragm was examined. A month or more after feeding, the surviving rats were killed, and their diaphragms were examined. Through an oversight no control animals were fed with unfrozen meat from the five hogs from which the meat was obtained for use in this set of experiments (77 to 87). In view of the undoubted viability of the trichinae in these hogs, however, as determined by the fact that the trichinae obtained from digested unfrozen meat were practically all active, very lively, and quite normal in all respects, this omission is not of great importance.

In the next series of experiments (88 to 90), meat was taken from the shoulders of seven naturally infested hogs slaughtered during December,

1914, and was inclosed on January 17, 1915, in three 1-pound cans ( $5\frac{1}{2}$  by  $2\frac{3}{4}$  inches), each can containing meat from all seven hogs. The shoulders after slaughter of the hogs were kept in a cooler at a temperature a few degrees above  $32^{\circ}$  F., except during the time when they were in transit between Chicago and Washington. Five of the seven hogs were the same as those from which the meat for experiments 77 to 87 was taken. On January 18 the three cans were placed in three freezers in New York City where they remained until February 1, a period of 14 days or, to be exact, 13 days, 23 hours. The temperature of the freezers as determined by thermometers compared with a standard thermometer during this period was  $4^{\circ}$  to  $7^{\circ}$ ,  $8^{\circ}$  to  $11^{\circ}$ , and  $14^{\circ}$  to  $16^{\circ}$  F., respectively (four readings daily). After removal from the freezers the cans were allowed to thaw at ordinary temperatures and were received for examination at the Washington laboratory on February 4.

The same routine as to the examination and feeding of experimental animals was followed as in the preceding experiments (77 to 87) except that the digesting fluid used contained only 5 gm. of sodium chlorid to each 1,000 c. c. of water, instead of 6 gm. In this case, as in the preceding set of experiments, no control animals were fed, but it happened that the test animals fed with the meat exposed to the temperature of  $14^{\circ}$  to  $16^{\circ}$  F. became infested, so that they served as a control upon those fed with meat exposed to the lower temperatures.

In the series of experiments numbered 91 to 126, the meat used was taken from six hogs slaughtered in Chicago prior to March 2, 1915, and found to be trichinous on microscopic examination. A shoulder from each of these hogs was sent in the fresh condition to Washington where it was retained in a cooler slightly above  $32^{\circ}$  F. to provide material for control examinations and feedings. The meat for the freezing experiments was inclosed in thirty-six 1-pound tin cans ( $5\frac{1}{2}$  by  $2\frac{3}{4}$  inches), some from each of the 6 hogs being placed in each can, so that each can contained a mixture of approximately equal portions of meat from all the hogs. On March 2, twelve of the cans were placed in a freezer maintained at a temperature of about  $5^{\circ}$  ( $5^{\circ}$  to  $6.5^{\circ}$ ), 12 in a freezer maintained at a temperature of about  $10^{\circ}$  ( $9^{\circ}$  to  $13^{\circ}$ ), and 12 in a freezer maintained at a temperature of about  $15^{\circ}$  ( $13.5$  to  $15^{\circ}$ ). After 10 days—on March 12—a can was removed from each of the 3 freezers and sent by mail to the Washington laboratory. The next day 3 more cans were removed as before, and so forth, the last cans being removed on March 25, after 23 days' exposure to cold. None was removed March 14 or 21, or 12 and 19 days, respectively, after they were placed in the freezers. The thermometers in these freezers, which were afterwards compared with a standardized thermometer, were read three times daily.

The same routine examination was followed as in experiments 77 to 90, described above, the formula of the digestant fluid being that used

in experiments 78 to 90—i. e., water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm.; sodium chlorid, 5 gm. A mixture of unfrozen meat from the six hogs was used in control examinations. As in the preceding experiments, five rats were fed meat from each can, following the same routine. Control animals were fed on June 15 with unfrozen meat from the six hogs which had been kept several months (since March) in a cooler. Meat from each hog was fed to two rats, 20 gm. being given to each two rats, an average of 10 gm. per rat.

In experiment 127, some meat from an artificially infested hog (the same hog from which meat was obtained in experiments 72 to 76) was inclosed in a half-pound tin can, which was placed in the center of a box 28 by 19 by 6½ inches containing about 100 pounds of pork trimmings. The box was placed in a freezer in Chicago, where it remained for 57 days, during which time the temperature as recorded by a thermometer afterwards compared with a standardized thermometer varied between 10° and 13° F. (three readings daily). After removal from the freezer the box was allowed to thaw for two days. The can was then removed and sent to the Washington laboratory. The same routine as to the examination and feeding of test animals was followed as in experiments 91 to 126.

There were no satisfactory control test animals in experiment 127, as the rats fed as controls in experiments 72 to 76, which would have served as controls in this experiment, were not fed until nearly eight months had elapsed since the slaughter of the hog from which the meat was obtained. No infestation resulted in these animals; the trichinæ were evidently all dead. Examination of some of the meat about six weeks later showed that the trichinæ were dead and disintegrated. The trichinæ, however, that were examined after artificial digestion of unfrozen meat from this hog as late as three weeks after slaughter appeared perfectly normal and were quite lively, and there is little doubt that control animals would have been infested if they had been fed early enough.

See Tables I and II for the results of these experiments.

TABLE I.—Results of examinations and feeding tests in refrigeration experiments with larvae of *Trichinella spiralis*

Ex- peri- ment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of day 5.	Examination of trichinae		Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)	
					From frozen meat.	From unfrozen meat (controls).	Fed frozen meat.	Fed unfrozen meat (con- trols).				
								Posi- tive	Nega- tive			
										(a)		(m)
(a)	(b)	(c)	(d)	(e)	(f) <sup>a</sup>	(g) <sup>b</sup>	(h) <sup>c</sup>	(i)	(k)	(l)	(m)	
1	1 rat. . .	Carcass. . .	-3 to -10	6	10	0			0	1		(k) Guinea pig;
2	do. . .	Vial. . .	4 to 10	(b)	10	0						(k) Guinea pig; (l, m) same as No. 8.
3	do. . .	Carcass. . .	13 to 15	2	12	100			0	1	0	Do.
4	1 rabbit . .	Vial. . .	-6	(c)	4	0			0	1	0	Do.
5	do. . .	do. . .	-6	(d)	2	0			0	1	0	(k) Guinea pig; (l, m) same as No. 8.
6	do. . .	do. . .	-6		2	100						(k, l) Guinea pigs; (l) slightly infested.
7	1 rat . . .	Carcass. . .	13 to 15	5	184	24	41	95	0	1	1	(f) Mostly sluggish; (k) guinea pig; (l, m) same as No. 8.
8	1 rabbit. . .	Leg do. . .	-2 to +3	2	209	2		100	0	1	1	(k) Guinea pig; (l, m) same as No. 8.
9	do. . .	Port of car- cass. . .	-2 to +3	5	656	0	104	100	0	1	0	(f) Nearly all very lively; (g, h) same as No. 10; (i) guinea pig slightly infested; (l, m) same as No. 8.
10	do. . .	do. . .										(k) Guinea pig; (l, m) same as No. 8.
11	do. . .	do. . .	11 to 15	6	80	81	104	100	1	0	1	(g, h) Same as No. 13; (k) guinea pig; (l, m) same as No. 8.
12	do. . .	do. . .	-2 to +3	6	275	1—	(?)	100	0	1	1	Do.
13	do. . .	do. . .	-1 to -2	5	433	0	37	100	0	1	1	(k) Guinea pig; (l, m) same as No. 8.
14	do. . .	do. . .	-2 to +3	8	200	0	37	100	0	1	1	(e) In water 4 days after digestion.
15	do. . .	do. . .	13 to 15	12	64	0	26	100	0	1	1	(c) In water 4 days after digestion; (f) 4 out of 7 very sluggish; (g, h, i, m) same as No. 16.
16	do. . .	do. . .	4 to 14	5	25	0	152	14	0	1	1	(c) In water 3 days after digestion; (f) very sluggish; (g, h, i, m) same as No. 16
17	do. . .	do. . .	12 to 14	5	100	7	152	14	0	1	1	(g, h, i, m) same as No. 16
18	do. . .	do. . .	14 to 22	5	42	5	152	14	0	1	1	(g, h, i, m) Same as No. 19.
19	1 rat. . .	do. . .	4 to 14	5	70	0	20	100	0	1	1	(l, m) Same as No. 19.
20	do. . .	do. . .	12 to 14	5	73	14	20	100	0	1	1	Do.
21	1 rabbit . .	do. . .	4 to 14	6					0	2	1	
21a	do. . .	do. . .	12 to 11	6					0	2	1	

<sup>a</sup> Percentages in columns (f) and (h) are expressed in the nearest whole numbers, except that percentages over 99.5 are expressed as 99.5 and less than 0.5 as 1—.

<sup>b</sup> 10 minutes.

<sup>c</sup> 20 minutes.

<sup>d</sup> 20 minutes.

TABLE I.—Results of examinations and feeding tests in refrigeration experiments with larvae of *Trichinella spiralis*—Continued

Ex- per- iment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinae.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (con- trols).		
					Num- ber ex- amined.	Percent- age active.	Percent- age active.	Num- ber ex- amined.	Posi- tive.	Nega- tive.	Posi- tive.	Nega- tive.	
22	1 rat....	Part of car- cass.	14 to 22 °F.	10	58	31	168	100	0	1	1	0	(f) Very sluggish; (l, m) same as No. 19.
22a	1 rabbit	do....	14 to 22	10	...	...	...	...	0	2	1	0	(l, m) Same as No. 16.
22b	do....	do....	12 to 14	10	...	...	...	...	0	2	1	0	Do.
23	1 hog...	Carcass....	14.5 to 15.5	7	476	98	208	97	0	0	2	1	(i) Four heavily, 2 moderately infested; (l, m) same as No. 34.
24	do....	do....	14.5 to 15.5	9	216	99+	120	100	6	0	2	1	(i) Four heavily infested; 1 degree of infestation not recorded; 1 guinea pig slightly infested; (l, m) same as No. 34.
25	do....	do....	14.5 to 16.5	10	180	59	182	98	0	4	2	1	(f) Sixty-three out of 107 sluggish; (k) 6 fed, 1 died after first feeding, another lost from cage; meat (loin) fed very lightly infested; (l, m) same as No. 34.
26	do....	do....	14.5 to 16.5	11	273	76	63	87	2	4	2	1	(i) One hundred and forty-five out of 208 sluggish; (i) slightly infested; (l, m) same as No. 34.
27	do....	do....	14.5 to 16.5	12	107	95	61	99	0	6	2	1	(f) Fifty-seven out of 102 sluggish; (l, m) same as No. 34.
28	do....	do....	14.5 to 16.5	13	59	42	61	99	0	6	2	1	(i) Twenty out of 25 sluggish; (g, h) same as No. 27; (l, m) same as No. 34.
29	do....	do....	14.5 to 16.5	14	...	...	...	...	6	0	2	1	(i) Three moderately, 2 slightly, 1 heavily infested; (l, m) same as No. 34.
30	do....	do....	14.5 to 16.5	16	102	74	99	99	2	1	2	1	(i) Slightly infested; (l, m) same as No. 34.
31	do....	do....	14.5 to 16.5	18	122	99	99	99	2	1	2	1	(g, h) Same as No. 30; (i) slightly infested; (l, m) same as No. 34.
32	do....	do....	14.5 to 16.5	21	135	18	123	80	0	3	2	1	(e) Meat digested 2 days; (g) meat from another car- cass digested 2 days; (l, m) same as No. 34.
33	do....	do....	14.5 to 16.5	22	110	84	200	100	0	3	2	1	(f) Fifty-three out of 99 sluggish; (g) meat from another carcass; (k) guinea pigs; (l, m) same as No. 34.
34	do....	do....	14.5 to 16.5	24	...	...	...	...	0	3	2	1	(i) Heavily infested.
35	do....	Half of car- cass.	8.5 to 9.5	5	267	16	80	100	1	2	2	1	(l, k) Guinea pigs; (i) lightly infested; (l, m) same as No. 41.
36	do....	do....	8.5 to 9.5	6	452	2	75	100	0	3	2	1	(f) Sluggish; (k) guinea pigs; (l, m) same as No. 41.

No.	Sex	Age	Date	Weight	Measurements	Remarks
37	do.	8-5 to 10-5	392	100	100	(i) Very sluggish; (l, m) same as in No. 41.
38	do.	8-5 to 10-5	298	123	86	(c, g) Meat digested 2 days.
39	do.	8-5 to 10-5	731	1—	100	(i) Very sluggish; (l, m) same as No. 41.
40	do.	8-5 to 10-5	701	1—	200	(i) One out of 3 sluggish, 2 very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
41	do.	8-5 to 10-5	221	14	100	(g, h) Same as No. 35; (i) 2 moderately, 1 slightly infested; (l, m) same as No. 41.
42	do.	5 to 7	159	0	75	(g, b) Same as No. 36; (k) guinea pigs; (l, m) same as No. 41.
43	do.	5 to 8	449	1—	100	(i) Very sluggish; (g, h) same as No. 37; (l, m) same as No. 41.
44	do.	5 to 8	326	0	123	(e) Meat digested 2 days; (g, h) same as No. 38.
45	do.	5 to 8	603	1—	200	(i) Very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
46	do.	5 to 8	517	0	300	(g, h) Same as No. 39; (l, m) same as No. 41.
47	do.	5 to 8	354	0	37	(i) Old infection, encysted trichinae in diaphragm; rat died 7 days after first feeding; (l, m) same as No. 41.
48	do.	5 to 8	230	3	37	(b) Sediment of digested meat in small vial.
49	rabbit	7 to 10	12	100	100	(b) Sediment of digested meat in small vial; (f) sluggish; (g, h) same as No. 49.
50	do.	10 to 13	105	71	100	(i) Heavily infested. Do.
51	hog	10 to 12	100	100	100	(i) Heavily infested; (g, h) same as No. 53; (k) died 3 days after first feeding.
52	do.	7 to 12	100	95	93	(i) Heavily infested.
53	do.	9 to 12	90	29	93	(i) Three heavily infested, 3 died early, few trichinae found.
54	do.	9 to 12	71	21	100	(i) Heavily infested, one very heavily infested.
55	do.	9 to 12	62	46	500	(g, h) Same as No. 51; (i) 5 rats fed, 1 died, eaten by others, 1 slightly infested, 2 heavily infested, 1 number of trichinae not recorded.
56	Carcass	10 to 11	400	46	99	(i) Slightly infested; (k) died 4 days after first feeding.
57	do.	10 to 12	105	71	100	(i) Heavily infested.
58	do.	9 to 12	100	100	100	(i) Three heavily infested, 3 died early, few trichinae found.
59	do.	9 to 12	100	100	100	(i) Heavily infested, one very heavily infested.
60	do.	9 to 12	100	100	100	(g, h) Same as No. 51; (i) 5 rats fed, 1 died, eaten by others, 1 slightly infested, 2 heavily infested, 1 number of trichinae not recorded.
61	do.	9 to 12	100	100	100	(i) Slightly infested; (k) died 4 days after first feeding.
62	do.	9 to 12	100	100	100	(i) Heavily infested.
63	do.	9 to 12	100	100	100	(i) Three heavily infested, 3 moderately infested.
64	do.	9 to 12	100	100	100	(g, h) Same as No. 51; (i) 3 heavily, 1 slightly infested, 1 number of trichinae not recorded;
65	6 hogs	10 to 13	523	31	127	(g, h) Same as No. 51; (i) 1 trichinae in each; (k) 1 died 1 day after first feeding.
66	do.	10 to 13	578	3	98	(g, h) Same as No. 51; (i) 1 trichinae in each; (k) 1 died 1 day after first feeding.
67	do.	10 to 13	578	3	98	(i) Very slightly infested; (l) all but 1 heavily infested.
68	do.	10 to 13	578	3	98	(b) Meat after thawing cured with salt 2 days before examination and feeding; (i) very slightly infested, 4 trichinae in each; (l, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
69	do.	10 to 13	578	3	98	(k) Five rats fed, 1 lost from cage; (l) 2 heavily, 3 slightly infested.
70	do.	10 to 13	578	3	98	(i) Heavily infested, 5 rats fed, 1 lost from cage; (l, m) same as No. 65.

**b 30 minutes.**

**2 25 minutes.**



TABLE I.—Results of examinations and feeding tests in refrigeration experiments with larvae of *Trichinella spiralis*.—Continued

Ex- peri- ment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinae				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (con- trols).		
					Num- ber ex- amined	Percent- age active.	Num- ber ex- amined.	Percent- age active.	Posi- tive.	Nega- tive.	Posi- tive.	Nega- tive.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
68	6 hogs...	100 pounds...	- 9 to 0	10	285	1	1,118	99	1	3	28	1	(l) One out of 2 sluggish; (i) 3 trichinae; (i, k) 5 rats fed, 1 died early not examined; (l, m) same as No. 65.
69	...do....	1/2 pound	- 9 to 0	10	221	0	1,118	99	0	5	28	1	(g, h) Same as No. 68; (i, m) same as No. 65.
70	...do....	100 pounds	- 9 to 0	14	1,442	1	436	99+	0	4	28	1	(g, h) Same as No. 68; (i, m) same as No. 65.
71	...do....	1/2 pound....	10 to 12	17	348	54	155	99	1	2	28	1	(g, h) Same as No. 68; (i, m) same as No. 65.
72	1 hog....	...do....	- 9 to 0	15	83	0	100	99	0	5	0	4	(f) Less active than normal, 33 out of 188 very sluggish; (i, k) 5 rats fed, 2 died, eaten by others; (i) 4 trichinae; (l, m) same as No. 65.
73	...do....	...do....	10 to 12	15	117	76	100	99	1	4	0	4	(l, m) Meat not fed until nearly 8 months after slaughter.
74	...do....	100 pounds...	- 9 to + 2	18	600	0	198	100	0	4	0	4	(f) Less active than normal, 33 out of 89 very sluggish; (i) identity questionable, numerous well-encysted trichinae 25 days after first feeding; (g, h, l, m) same as No. 72.
75	...do....	1/2 pound....	- 9 to + 2	20	386	0	198	100	0	4	0	4	(g, h) Five rats fed, 1 died, eaten by others; (l, m) same as No. 72.
76	...do....	...do....	10 to 13	20	669	48	198	100	0	4	0	4	(g, h) Same as No. 74; (l, m) same as No. 72.
77	...do....	100 pounds	- 2 to + 5	15	153	0	103	90+	0	5	...	...	(f) Mostly less active than normal, 219 out of 319 sluggish; (g, h) same as No. 74; (l, m) same as No. 72.
78	...do....	1 pound...	12 to 16	17	139	50	103	99+	0	5	...	...	(f) Mostly quite lively; (g, h) same as No. 79.
79	...do....	380 pounds	- 2 to + 5	21	80	0	138	100	0	5	...	...	(f) Same as No. 79.
80	...do....	200 pounds	- 2 to + 2	14	122	0	138	100	0	5	...	...	(f) Mostly sluggish; (g, h) same as No. 79.
81	...do....	1 pound	13 to 15	17	304	65	138	100	0	5	...	...	(f) Mostly sluggish; (g, h) same as No. 79.
82	...do....	100 pounds.	- 2 to + 1	15	351	0	138	100	0	5	...	...	(f) Mostly sluggish; (g, h) same as No. 79.
83	...do....	1 pound	13 to 15	17	243	42	138	100	0	5	...	...	(f) Mostly sluggish; (g, h) same as No. 79.
84	...do....	100 pounds	- 2 to + 2	17	80	0	138	100	0	5	...	...	(f) Same as No. 83; (i) died 4 days after first feeding, 1 live, 2 dead larvae in intestine, undeveloped.
85	...do....	1 pound	13 to 15	18	86	38	138	100	0	5	...	...	
86	...do....	100 pounds	- 2 to + 2	15	291	0	120	100	1	4	...	...	

(i) One out of 2 sluggish; (j) 3 trichinae; (i, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.  
 (g, h) Same as No. 68; (l, m) same as No. 65.  
 (f) Very sluggish; (k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.  
 (i) Less active than normal, 79 out of 188 very sluggish; (i, k) 5 rats fed, 2 died, eaten by others; (j) 4 trichinae; (l, m) same as No. 65.  
 (l, m) Meat not fed until nearly 8 months after slaughter.  
 (f) Less active than normal, 38 out of 89 very sluggish; (i) identity questionable, numerous well-encysted trichinae 25 days after first feeding; (g, h, l, m) same as No. 72.  
 (k) Five rats fed, 1 died, eaten by others; (l, m) same as No. 72.  
 (g, h) Same as No. 74; (l, m) same as No. 72.  
 (f) Mostly less active than normal, 210 out of 310 sluggish; (g, h) same as No. 74; (l, m) same as No. 72.  
 (f) Mostly quite lively; (g, h) same as No. 77.  
 (g, h) Same as No. 79.  
 (f) Mostly quite lively; (g, h) same as No. 79.  
 (g, h) Same as No. 79.  
 (f) Mostly sluggish; (g, h) same as No. 79.  
 (f) Mostly sluggish; (g, h) same as No. 84.  
 (g, h) Same as No. 84; (i) died 4 days after first feeding, 1 live, 2 dead larvae in intestine, undeveloped.

87	...do....	1 pound....	13 to 15	18	205	67	120	100	0	5	.....	(i) Mostly quite lively, but paler than normal; (g, h) same as No. 84.
88	7 hogs....	.....do....	4 to 7	14	744	1	143	100	0	5	5	(f) Very sluggish; (i) test rats Exp. No. 90, serve as controls for Exp. Nos. 88, 89.
89	...do....	.....do....	8 to 11	14	121	98	43	100	2	3	5	(f) Not so lively as paler than normal; (i) slightly infested; (l, m) same as No. 88.
90	...do....	.....do....	14 to 16	14	42	100	43	100	5	0	.....	(g, h) Same as No. 89; (i) heavily infested.
91	6 hogs....	½ pound....	5 to 6.5	10	213	0	150	100	0	5	11	(i) Eight heavily infested, degree of infestation not recorded in 3; (m) killed 4 days after feeding, only small portion of intestine examined.
92	...do....	.....do....	5 to 6.5	11	300	0	150	100	0	5	11	(g, h, l, m) Same as No. 91.
93	...do....	.....do....	5 to 6.5	13	151	0	100	100	0	5	11	(l, m) Same as No. 91.
94	...do....	.....do....	5 to 6.5	14	204	2	55	100	0	5	11	(i) Very sluggish; (l, m) same as No. 91.
95	...do....	.....do....	5 to 6.5	15	202	0	100	100	0	5	11	(l, m) Same as No. 91.
96	...do....	.....do....	5 to 6.5	16	159	0	133	90	0	5	11	(e, g) Digested nearly 2 days; (l, m) same as No. 91.
97	...do....	.....do....	5 to 6.5	17	267	0	150	100	0	5	11	(l, m) Same as No. 91.
98	...do....	.....do....	5 to 6.5	18	139	3	150	100	0	5	11	(i) Very sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
99	...do....	.....do....	5 to 6.5	20	180	0	100	100	0	5	11	(l, m) Same as No. 91.
100	...do....	.....do....	5 to 6.5	21	.....	.....	.....	.....	0	5	11	Do.
101	...do....	.....do....	5 to 6.5	22	340	0	200	100	0	5	11	Do.
102	...do....	.....do....	5 to 6.5	23	105	Many.	(?)	100	0	5	11	Do.
103	...do....	.....do....	10.5 to 13	10	(?)	.....	150	100	5	0	11	(g, h) Same as No. 91; (i) heavily infested, 1 very heavily; (l, m) same as No. 91.
104	...do....	.....do....	10.5 to 13	11	(?)	46	150	100	5	0	11	(g, h) Same as No. 91; (i) 4 heavily infested, 1 slightly infested; (l, m) same as No. 91.
105	...do....	.....do....	10.5 to 13	13	.....	33	100	100	5	0	11	(f) Very sluggish; (i) 1 heavily infested, others with 1, 22, 35, and 126 trichinae; (g, h) same as No. 93; (l, m) same as No. 91.
106	...do....	.....do....	10.5 to 13	14	50	90	55	100	3	2	11	(f) Less active than normal; (i) 20, 7, and 4 trichinae; (g, h) same as No. 91; (l, m) same as No. 91.
107	...do....	.....do....	10.5 to 13	15	36	78	100	100	0	5	11	(f) Less active than normal; (g, h) same as No. 95; (l, m) same as No. 91.
108	...do....	.....do....	10.5 to 13	16	152	5	153	90	0	5	11	(f) Very sluggish, digested nearly 2 days; (g, h) same as No. 96; (l, m) same as No. 91.
109	...do....	.....do....	10.5 to 13	17	26	29	150	100	0	4	11	(f) Sluggish; (k, l) 3 rats fed, 1 died, eaten by others; (g, h) same as No. 97; (l, m) same as No. 91.
110	...do....	.....do....	10.5 to 13	18	35	31	150	100	0	5	11	(f) Sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
111	...do....	.....do....	9.5 to 13	20	67	18	100	100	0	5	11	(f) Less active than normal; (g, h) same as No. 99; (l, m) same as No. 91.
112	...do....	.....do....	9 to 13	21	.....	.....	.....	.....	0	5	11	(l, m) Same as No. 91.
113	...do....	.....do....	9 to 13	22	(?)	(?)	200	100	0	5	11	(f) Sluggish; (g, h) same as No. 101; (l, m) same as No. 91.
114	...do....	.....do....	9 to 13	23	74	34	(?)	100	0	5	11	(f) Sluggish; (g, h) same as No. 102; (l, m) same as No. 91.
115	...do....	.....do....	14.5 to 15	10	(?)	Many.	150	100	5	0	11	(f) Commonly less active than normal; (g, h) same as No. 91; (i) 4 heavily, 1 very heavily infested; (l, m) same as No. 91.
116	...do....	.....do....	13.5 to 15	11	(?)	.....	150	100	5	0	11	(f) Commonly less active than normal; (g, h, l, m) same as No. 91.
						.....	.....	.....	.....	.....	.....	(i) Four heavily, 1 very heavily infested.

TABLE I.—Results of examinations and feeding tests in refrigeration experiments with larvae of *Trichinella spiralis*—Continued

Ex- peri- ment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinae.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)	
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (con- trols).			
					Num- ber ex- amined.	Percent- age active.	Num- ber ex- amined.	Percent- age active.	Posi- tive.	Nega- tive.	Posi- tive.	Nega- tive.		
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	
117	6 hogs...	½ pound	°F. 13.5 to 15	13	(?)	Many.	100	100	5	0	11	1		(i) Commonly less active than normal; (g, h) same as No. 93; (l, m) same as No. 91.
118	do...	do...	13.5 to 15	14	95	99	55	100	4	1	11	1		(i) Three heavily, 1 very heavily infested, 1 with 43 trichinae.
119	do...	do...	13.5 to 15	15	(?)	Many.	100	100	5	0	11	1		(i) Mostly quite lively; (i) 2 heavily infested, 2 with 9 and 3 trichinae; (g, h) same as No. 94; (l, m) same as No. 91.
120	do...	do...	13.5 to 15	16	175	26	153	99	5	0	11	1		(i) Mostly quite lively; (i) heavily infested; (g, h) same as No. 95; (l, m) same as No. 91.
121	do...	do...	13.5 to 15	17	(?)	Many.	150	100	4	1	11	1		(i) Very sluggish, digested 2 days; (i) 4 heavily infested, 1 with 4 trichinae; (g, h) same as No. 96; (l, m) same as No. 91.
122	do...	do...	13.5 to 15	18	(?)	do...	150	100	3	1	11	1		(i) Quite lively; (i) 2 heavily infested, 2 with 5 and 6 trichinae; (g, h) same as No. 97; (l, m) same as No. 91.
123	do...	do...	13.5 to 15	20	(?)	do...	100	100	4	1	11	1		(i) Quite lively; (g, h) same as No. 99; (i) slightly in- fested, 1, 7, 4, and 18 trichinae; (l, m) same as No. 91.
124	do...	do...	13.5 to 15	21	do...	do...	do...	do...	0	5	11	1		(l, m) Same as No. 91.
125	do...	do...	13.5 to 15	22	(?)	(?)	200	100	0	5	11	1		(i) Some quite lively; (g, h) same as No. 101; (l, m) same as No. 91.
126	do...	do...	13.5 to 15	23	(?)	(?)	(?)	100	3	2	11	1		(i) Fairly lively; (g, h) same as No. 102; (i) slightly in- fested, 12, 27, and 1 trichinae; (l, m) same as No. 91.
127	1 hog...	100 pounds	10 to 13	57	97	6	52	98	0	5	0	4		(i) Five out of 6 very sluggish, other 1 sluggish; (l, m) same as No. 72.

TABLE II.—Summary of results of refrigeration experiments with larvæ of *Trichinella spiralis* exposed to various temperatures

Exposure to about 15° F.				Exposure to about 10° F.				Exposure to about 5° F.				Exposure to about 0° F.			
Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.
3	2	+	...	50	(a)	+	...	49	(b)	—	...	4	(c)	—	—
7	5	+	...	35	5	+	...	2	(a)	—	...	5	8	—	—
17	5	+	...	11	6	+	...	16	5	—	...	6	8	—	—
15	5	+	...	36	6	+	...	19	5	—	...	8	2	+	...
20	5	+	...	56	6	+	...	42	5	+	+	9	3	+	...
21a	6	+	...	37	7	+	...	21	6	...	...	10	5	+	...
23	7	+	...	51	7	+	...	43	6	...	...	13	5	+	...
24	9	+	...	57	7	...	...	44	7	+	...	67	5	+	...
25	10	+	...	38	8	...	...	45	8	+	...	1	6	+	...
22	10	+	...	52	8	...	...	46	9	+	...	12	6	+	...
22a	10	+	...	58	8	+	...	47	10	...	...	14	8	...	...
22b	10	+	...	39	9	+	...	91	10	...	...	68	10	+	...
115	10	+	...	53	9	+	...	48	11	...	...	69	10	+	...
26	11	+	...	59	9	...	...	92	11	...	...	70	14	+	...
116	11	+	...	40	10	+	...	93	13	...	...	72	15	...	...
15	12	+	...	54	10	+	...	88	14	+	...	77	15	...	...
27	12	+	...	60	10	...	...	94	14	+	...	80	15	...	...
28	13	+	...	101	10	+	...	95	15	...	...	82	15	...	...
117	13	+	...	41	11	...	...	96	16	...	...	84	15	...	...
29	14	+	...	55	11	+	...	97	17	...	...	86	15	...	...
90	14	+	...	61	11	...	...	98	18	+	...	74	18	...	...
118	14	+	...	104	11	+	...	99	20	...	...	75	20	...	...
119	15	+	...	62	12	+	...	100	21	...	...	79	23	...	...
30	16	+	...	63	13	+	...	101	22	...	...				
120	16	+	...	105	13	+	...	102	23	...	...				
78	17	+	...	64	14	+	...								
81	17	+	...	89	14	+	...								
83	17	+	...	106	14	+	...								
121	17	+	...	73	15	+	...								
11	18	+	...	107	15	+	...								
85	18	+	...	108	16	+	...								
87	18	+	...	71	17	+	...								
122	18	+	...	109	17	+	...								
123	20	+	...	110	18	+	...								
32	21	+	...	95	19	+	...								
124	21	+	...	65a	19	+	...								
33	22	+	...	66	19	+	...								
125	22	+	...	76	20	+	...								
126	23	+	...	111	20	+	...								
34	24	...	—	112	21	...	...								
				113	22	+	...								
				114	23	+	...								
				127	57	+	...								

a 30 minutes.

b 25 minutes.

c 10 minutes.

d 20 minutes.

## RESULTS OF EXPERIMENTS

## EFFECTS OF VARIOUS LOW TEMPERATURES UPON THE VITALITY OF TRICHINÆ

In only one instance out of 34 experiments in which trichinous meat was exposed to temperatures of about 15° F. for periods ranging from 2 to 23 days were all of the trichinæ upon examination found to be inactive (experiment 15, 12 days). In most instances, although some were found to be inactive, a large proportion were commonly found to be active, not rarely as high as 98 to 100 per cent. In one case, even after 18 days' exposure (experiment 31), over 99 per cent of the trichinæ were found active on examination, and in another case after 22 days (experiment 33) 84 per cent were active.

In 38 experiments test animals were fed meat which had been exposed to about 15° F. for periods ranging from 5 to 24 days, with positive results—i. e., resultant infection—in 17 experiments and negative results in 21.

Some of the negative results were obtained in experiments in which the meat had been kept in the freezer for only 5 and 6 days; on the other hand, positive results were obtained from feeding meat which had been in the freezer for 23 days. Heavy infections were obtained from meat exposed as long as 18 days (experiment 122), but only slight infections resulted from meat kept in the freezer for 20 days or longer (seven experiments), and then only in two instances: In experiment 123 (20 days) one rat was negative, four slightly infested, and in experiment 126 (23 days) two rats were negative, three slightly infested.

From these results it appears that trichinous meat commonly fails to produce infection after exposure to temperatures of about 15° F. for periods of 5 to 24 days, notwithstanding the fact that many trichinae remain alive and are quite lively when thawed out after such exposure. Failure to infect is probably because, first, of a reduction in the number of live trichinae and, second, of a reduction in the vitality of those that remain alive. It may be concluded that although a temperature of 15° F. has an injurious action upon the vitality of trichinae, this temperature is uncertain in its effects and that meat exposed to a temperature of 15° F. for as long as 23 days is still liable to produce infection. These results correspond to those obtained by Schmidt, Ponomarer, and Savelier (1915) who concluded from their experiments that a temperature of -9° C. (+15.8° F.) is sometimes fatal to trichinae, but not always and that the results of exposure to this temperature are variable and uncertain.

The same authors also found that a temperature of -6° (+21.2° F.) has comparatively little effect upon trichinae exposed to it for a period of 10 days.

Trichinae were found to be alive upon examination in 34 out of 35 experiments in which trichinous meat was exposed to temperatures of about 10° F. for periods varying between 30 minutes and 57 days, all but one of the experiments having to do with periods of 5 to 23 days. In the one case in which all of the trichinae were found to be dead (experiment 38) the meat had been artificially digested for 2 days in preparation for examination instead of less than 24 hours as usual, which is the probable explanation why none was found alive. Although there were no striking differences in the percentages of trichinae found alive as compared with the findings in the experiments in which meat was exposed to temperatures of about 15°, it was frequently noted that they were less lively than normal, commonly sluggish. In 20 of the experiments a record was made of the degree of activity and it was noted that in 19 of these the trichinae were sluggish, or at least less lively than

normal, and that in the twentieth they were nearly all very lively (experiment 11, 6 days' exposure). It was quite noticeable in the examinations that the activity of the trichinae was generally much more impaired than in the case of meat exposed to 15°.

In 41 out of the total of 43 experiments in which meat was exposed to temperatures of about 10° F., test animals were fed, the results being positive in 22 cases, negative in 19. In one of the latter (experiment 73) one out of five rats was found to be heavily infested, but there is a question as to the identity of this rat; furthermore, the trichinae were too far advanced in development to have resulted from meat fed at the time the rats belonging to this lot were fed. In feedings with meat exposed to temperatures of about 10° for 13 days or less, heavy infestations were commonly produced, but in 17 experiments with meat exposed 14 to 23 days and in one with meat exposed 57 days the results of feeding were either negative or, if infection was produced, it was slight. In only 4 of these 18 experiments did any of the test animals become infested. In experiment 106 (14 days) three rats were slightly infested, two negative; in experiment 71 (17 days) one was very slightly infested (four trichinae in diaphragm), two negative; in experiment 65 (19 days) four were very slightly infested, one negative; and in experiment 65a (19 days) two were very slightly infested (four trichinae in the diaphragm of each), two negative.

Summarizing the results of the experiments with meat exposed to temperatures of about 10° F. it may be noted that trichinae have been found to survive in meat exposed for as long as 57 days, though in that case only a small percentage, and those only sluggishly active, and that some survived in nearly all cases, their numbers and vitality, however, having been so reduced that after 14 days' exposure either no infection resulted in test animals or, if infection resulted, it was very slight. Evidently, therefore, the effects of a temperature of 10° upon the vitality of trichinae are decidedly more pronounced than those of a temperature of 15°.

Twenty-five experiments were carried out in which trichinous meat was exposed to temperatures of about 5° F.; and in 23 of these, examinations were made of the trichinae after thawing. In only six instances were live trichinae found. In experiment 42 (5° to 7° for 5 days) 14 per cent of the trichinae were found to be alive, degree of activity not recorded. The number of live trichinae found in the five other experiments ranged from less than 1 per cent to 3 per cent, and they were all very sluggish (experiments 44, 46, 88, 94, 98), the periods of exposure to cold being 7, 9, 14, 14, and 18 days, respectively.

Test animals were fed in 23 experiments. No infections resulted except in experiment 42, just referred to. In this experiment three rats were fed and two became moderately and one slightly infested.

The results of these experiments show that temperatures of about 5° F. have a profound effect upon the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are extremely unlikely to occur, none having resulted in any case in which test animals were fed meat exposed to temperatures of about 5° for periods ranging from 6 to 23 days (19 experiments). In view of the results of experiment 68, however, in which the temperature was -9° to 0° and the period of exposure 10 days, it may be concluded that slight infections may sometimes result from meat exposed to 5° for as long as 10 days.

The results of the experiments with temperatures of about 5° F. correspond closely to those of Schmidt, Ponomarer, and Savelier (1915). These authors, however, found that in their experiments a temperature of -15° to -16° C. (3.2° to 5° F.) was always fatal to trichinæ and noted no exceptions such as were observed by the present writer.

In experiments in which trichinous meat was exposed to temperatures of about 0° F., but ranging as low as -10° in some instances, trichinæ were rarely found to be alive. However, 100 per cent were found to be alive in one experiment (No. 67) in which meat had been exposed to a temperature of -4° to 0° for 5 days, but in 15 experiments in which the period of exposure to cold ranged from 6 to 23 days trichinæ were found alive only in three instances and less than 1 per cent in each case (experiments 12, 68, and 70).

Test animals were fed in all but 1 of the 23 experiments with temperatures of about 0° F. Infection resulted in two instances. Four rats fed in experiment 67 (-4° to 0°, for 5 days) became heavily infested, and one out of four in experiment 68 (-9° to 0°, for 10 days) showed three trichinæ in the diaphragm, the three other rats being negative. In the latter case, as in the former, live trichinæ had been found by examination of the meat; less than 1 per cent, however, as compared with 100 per cent in the former, the results of the feeding tests thus as usual being quite consistent with the results of the examinations of artificially digested meat, though it was unusual for infection to result when the examination showed such a small percentage of live trichinæ as in experiment 68. In experiment 86 (-2° to +2°, for 15 days), in which no trichinæ were found alive on examination of artificially digested meat, the result of the feeding test is considered to have been negative, although one of the five test rats, which died four days after feeding, was found to have three trichina larvæ in the intestine, two of which were dead, whereas the other one exhibited feeble movements. None of these three larvæ, however, had undergone any development, and the four other test rats were negative, so that it seems quite proper to conclude that the viability of the trichinæ had been destroyed in the meat in question.

From the foregoing it appears that the results of exposing trichinous meat to temperatures of about 0° F. are similar to those produced by temperatures of about 5°—i. e., a few trichinæ may survive exposures to such temperatures for 6 days or more, but their vitality will be so greatly reduced that there is little likelihood of their causing infection, although, on the other hand, slight infections may result from meat exposed as long as 10 days.

A good example of the relative effects of different low temperatures upon the vitality of trichinæ is supplied by experiments 91 to 126. In these experiments approximately equal quantities of trichinous pork from the same source (mixture of meat from six hogs) were exposed for 10 to 23 days in three freezers at temperatures of about 15°, 10°, and 5° F., respectively, a can of meat being removed from each of the three freezers after 10 days' exposure, another after 11 days, and so on (no cans, however, being removed on the twelfth or nineteenth day). It will be observed from the recorded results (Tables I, II) that many of the trichinæ in the meat exposed to a temperature of about 15° survived, and up to the twentieth day of exposure were mostly quite lively after thawing. Some of those from meat exposed for 22 days were observed to be quite lively, and those which survived in meat exposed for 23 days were found to be fairly lively. From the results of the feeding tests there appeared to be a considerable reduction in the vitality of the parasites after 17 days' exposure, notwithstanding the survival of a large percentage. Most of the rats fed meat exposed to about 15° for 10 to 16 days became heavily infested, but the 17-day meat failed to infect one out of five rats, and only two of the four others became heavily infested, the 18-day meat failed to infect one out of five, the 20-day meat failed to infect one, the four others becoming only slightly infested, none of the rats fed 21- and 22-day meat became infested, and the 23-day meat failed to infect two and produced only light infestations in the three others.

In the case of the meat exposed to a temperature of about 10° F. it was observed that the trichinæ which survived were relatively less numerous, as a rule, than in the case of the meat exposed to about 15°, and it was generally noted that they were less active than normal, or sluggish, sometimes very sluggish. The test rats, fed meat exposed for 10 days, all became heavily infested, all five fed 11-day meat became infested, but one was only slightly infested, all five fed 13-day meat became infested, but only one was heavily infested, three out of five fed 14-day meat became infested, but these only slightly, and none of the rats fed meat exposed to about 10° for 15 days or longer became infested. In this series, therefore, there was apparently a considerable reduction in the infectiousness of the meat beginning with that exposed for 13 days, and after 2 days more the infectiousness became nil.

Practically none of the trichinæ in the meat exposed to a temperature of about 5° F. (experiments 91 to 102) survived; although living trichinæ



were observed in meat exposed for 14 and 18 days (2 and 3 per cent, respectively), these were very sluggish. Furthermore, none of the test rats in this series became infested.

The results of the three sets of experiments just cited demonstrate quite clearly that a temperature of 10° F. is more effective in destroying the vitality of trichinæ than a temperature of 15°, and that a temperature of 5° is still more effective, illustrating the general rule established by the investigations recorded in the present paper, that within certain limits the effect upon the vitality of trichinæ becomes more pronounced as the temperature of refrigeration is lowered. It has also apparently been established that the increase in effectiveness is not uniform with the decrease in the temperature, but that somewhere in the neighborhood of 10° a critical point is reached, below which there is a sudden increase in the effectiveness of refrigeration.

Summarizing the results of the various experiments with a view to their practical application, inasmuch as very few trichinæ have been found to survive an exposure of more than 10 days to a temperature of 5° F., or lower, and as the few surviving have shown only very slight activity, and as, moreover, trichinous meat exposed to temperatures of 5° or lower has rarely produced infestation, and has never (in repeated trials) produced infestation when the period of exposure was more than 10 days, it may be concluded that meat exposed to a temperature not higher than 5° for a period of 20 days will no longer contain viable trichinæ, 10 days in this 20-day period being allowed as a margin of safety. It may be further concluded that, so far as our present knowledge goes, temperatures of 10° and higher are too uncertain in their effects upon the vitality of trichinæ to justify the use of refrigeration at such temperatures as a means of rendering trichinous meat innocuous.

#### CHANGES PRODUCED IN TRICHINA LARVÆ BY EXPOSURE TO LOW TEMPERATURES

Low temperatures (15° F. and lower) not only destroy the vitality of some or all of the trichinæ which are exposed to those temperatures but they produce changes in the tissues of the parasites, which are apparent under the microscope. These changes in appearance are associated with reductions in the activity of the trichinæ and with losses in their vitality.

Trichinæ from artificially digested unfrozen meat when examined under the microscope in water, or preferably in a physiological salt solution are found to be tightly coiled, becoming very lively when they are warmed to body temperature and continuing their lively movements as the temperature increases up to about 50° or 52° C. when they become sluggish and finally cease movement and die when the temperature rises a few degrees higher. The esophageal cellular body of the normal trichina has a bright yellowish brown color, and exhibits a certain granulation of the protoplasm; the nuclei of the cells are apparent as small,

clear, spherical bodies, seemingly of a vesicular nature. The gonad (ovary or testis) forms a continuous mass of cells closely pressed together, intercellular divisions and nuclei being indistinct in the living specimen. The body cavity forms a thin but distinct space between the internal organs and the parietal wall. In short, the normal living trichina larva freed from its capsule by artificial digestion presents a sharp clear-cut bright appearance which is quite characteristic but difficult to describe.

The changes shown by the trichinae from artificially digested meat in experiments 118, 106, and 94 are typical of those produced by the exposure of trichinous meat to various low temperatures. In these instances the temperatures were  $13.5^{\circ}$  to  $15^{\circ}$ ,  $10.5^{\circ}$  to  $13^{\circ}$ , and  $5^{\circ}$  to  $6.5^{\circ}$  F., respectively, and the period of exposure 14 days in each case. The meat was all of the same origin—i. e., from six hogs, mixed together, portions of about half a pound being inclosed in tin cans and placed in freezers maintained at the temperatures stated. The cans were removed at the end of 14 days and the meat allowed to thaw at ordinary temperatures. Two days after removal from the freezers the meat from each can was ground up, digested overnight in an artificial gastric juice, washed and sedimented in a 0.6 per cent salt solution and the trichinae thus obtained subjected to examination. As usual, for the purpose of controlling the results of these processes upon the frozen meat, unfrozen meat from the same carcasses was digested, washed, and examined in exactly the same manner.

Out of 95 trichinae from the meat which had been exposed to a temperature of  $13.5^{\circ}$  to  $15^{\circ}$  F. (No. 118), only one was inactive, this one being pale in color, and the nuclei in the cellular body having a solidified appearance. The 94 others were more or less tightly coiled when cold, and most of them were quite lively when warmed. The granulation of the protoplasm of the cellular body differed only slightly from normal, and its color was nearly normal; the nuclei showed commonly a small central point of more solid appearance than the remainder of the nucleus. The gonad either showed only slight changes from normal or the germ cells were rounded instead of being closely pressed together, this rounding of the cells occurring in only a part of or throughout the gonad. Two of the test rats in this experiment became heavily infested; one was negative; one showed 9 trichinae in the diaphragm; and one 3 trichinae in the diaphragm.

Fifty trichinae were examined from the meat which had been exposed to a temperature of  $10.5^{\circ}$  to  $13^{\circ}$  F. Of these, five were inactive, pale in color, their coils expanded so that they resembled a figure 6, and the nuclei of the cellular body of the esophagus were solidified. The 45 which were active were more or less tightly coiled when cold, some of them being quite lively when warmed. The color of the cellular body was rather paler than normal, the protoplasm abnormally granular, the nuclei either not apparent or exhibiting a solidified central portion. The cells of the

gonad were rounded instead of being closely pressed together as in the normal trichina. Two out of five test rats were negative, the three others contained 4, 7, and 20 trichinæ, respectively, in the diaphragm.

In experiment 94, in which the meat had been exposed to a temperature of 5° to 6.5° F., 204 trichinæ were examined, 199 of which were inactive, and only 5 of which showed any activity when warmed, this consisting of a very slight movement on stimulation with a needle point. The coils were expanded in the form of a figure 6, or in some instances formed a very loose spiral. The esophageal cellular body was very pale in color, granulation of the protoplasm very abnormal, nuclei solidified, quite different in appearance from the normal vesicular nucleus. The cells of the gonad were rounded and more or less dissociated. Five test rats fed in this experiment all failed to become infested.

The abnormal granulation of the cellular body referred to is difficult to describe, but it gives the protoplasm a distinctly different appearance from that of the cellular body of an unfrozen trichina, dull and dead-looking as compared with the bright appearance of the latter, the visible particles being much more numerous and smaller.

Comparison of the results of these three experiments and similar experiments shows not only that microscopically visible changes occur in the minute structure of trichinæ subjected to temperatures of 15° F. and lower, but that these changes are more pronounced in trichinæ subjected to about 10° than in those subjected to about 15°, and still more pronounced in trichinæ subjected to about 5°. These changes are evidently brought about by the low temperature, but in what way is not apparent. This problem probably belongs in the field of colloid chemistry. There occurs perhaps a precipitation of the colloids in the tissues of the trichina or some change in their nature which is more or less irreversible, according as the temperature is lower or higher and the period of exposure longer or shorter. In those cases in which the trichinæ were examined very soon after thawing of the meat (experiments 1 and 3, for example) it was quite evident from the shriveled appearance of the parasites that fluid had been extracted from them during their exposure to cold. Trichinæ thus shriveled absorb moisture after thawing and soon lose their shriveled appearance, again becoming active unless the temperature was too low and the period of exposure to cold too long continued. In some respects trichinæ which have been frozen at a low temperature (5° F.) resemble those which have been dried and then moistened again. Ordinary drying, however, destroys the vitality of trichinæ immediately, and the changes produced are much more marked than those produced by freezing. It is possible that the latter might be more closely simulated if the trichinæ were very gradually dried and the drying process stopped at the proper point. As yet, however, careful experiments along this line have not been carried out.

In view of the recent discovery by plant physiologists (see Bachmann, 1914) that sugar in plant tissues acts in some manner to protect them from the injurious effects of freezing so that the same species of plant is able to withstand a lower temperature when its tissues are loaded with sugar than when they contain only small quantities of this substance, it is of interest to note that larval trichinae contain a high percentage of glycogen.

Whatever may be the explanation of the destruction of the vitality of trichinae and of the changes brought about by exposure to cold, the investigations thus far carried out are sufficient to prove that trichinae when exposed to temperatures of 15° F. or lower undergo changes in their protoplasmic structure, and if the temperature is low enough and the exposure to cold continued long enough these changes become so pronounced and so well established that the vitality of all of the parasites is entirely destroyed.

#### VARIATIONS IN VITALITY OF TRICHINÆ

It is natural to expect that individual trichinae would vary in resistance to the effects of cold, and this was found to be the case. Some succumb much more quickly and at higher temperatures than others. In order to avoid misleading results on this account, meat was not used in the experiments unless heavily infested so that large numbers of trichinae might be available for study, considerable quantities were used, as a rule, for examination and for feeding tests, several test animals (four to six) being generally employed; and, commonly, mixed meat from several hogs was used so that the chances of including only feebly resistant trichinae in an experiment may be considered to have been reduced to a minimum in most cases.

#### QUANTITIES OF MEAT FROZEN

As already noted, various quantities of meat ranging from a gram or two up to nearly 400 pounds in weight were frozen in the various experiments. The rate of freezing and thawing, of course, varied with the quantity of meat, the change of temperature being rapid when small quantities, slow when large quantities were used. When very small quantities of meat or of fluid containing free trichinae were frozen and thawed within a few minutes (experiments 2, 4, 5, 6, 49, 50) the trichinae were apparently much more injuriously affected than when larger quantities of meat were subjected to similar temperatures for considerably longer periods of time. On the other hand, if the quantity of meat weighed half a pound or more, differences in the weight, and consequently in the rate of freezing and thawing, made no appreciable difference in the effect upon the vitality of the trichinae, as is quite evident from a comparison of the various experiments recorded in the tables. In short, it may be

stated that if the temperature to which trichinous meat is exposed is sufficiently low and the length of exposure sufficiently long, the trichinae are killed just as certainly when large quantities of meat are frozen as when small quantities (not less than half a pound) are frozen, variations in the rate of freezing and thawing dependent upon variations in the quantity of meat frozen being immaterial.

VARIATIONS IN LENGTH OF TIME AFTER REMOVAL FROM FREEZER BEFORE  
EXAMINING AND TESTING MEATS

In some cases examination of the trichinae from meat which had been frozen was made on the same day the meat was removed from the freezer or freezing mixture. When the meat was digested before examination, it was in some instances placed in the digesting fluid the same day the meat was removed from the freezer, but generally one or more days up to a maximum of 12 days elapsed before the meat was digested and examined, and a corresponding period before the feeding of test animals was begun.

Nearly all of the experiments were carried out in cold weather, and the meat after thawing, except when in transit to the laboratory, was kept in coolers or ice boxes until it was placed in a digesting fluid or fed to test animals, so that decomposition changes were slight.

In the majority of instances the meat was placed in digesting fluid in preparation for examination and the feeding of rats begun within four days after removal from the freezer, but longer periods appeared to have no pronounced effect upon the results. Certainly the lapse of time did not favor the revival of the trichinae. For example, in experiments 77, 80, 82, 84, and 86 the periods which elapsed between removal from the freezer (about 0° for 15 days) and the digestion of the meat were 12, 8, 8, 10, and 10 days, respectively; and between removal from the freezer and the first feedings of test animals, 13, 8, 8, 10, and 10 days, respectively, yet no trichinae were found alive on examination, and none of the test animals became infested. On the other hand, it did not seem that the lapse of time following removal from the freezer had much effect in reducing the vitality of surviving trichinae, though it is quite likely that the longer the period which elapses after trichinous meat is removed from the freezer the fewer the surviving trichinae will be, other things being equal. In experiments 126, 81, 83, 85, 87, and 78, the periods elapsing between removal from the freezer (about 15°, 17 to 23 days) and digestion of the meat were 6, 6, 6, 7, 7, and 9 days, respectively, and between removal from the freezer and the first feedings of test animals 4, 6, 6, 1, 7, and 10 days, respectively. A high percentage of trichinae were found to be alive in each case. In only one of the experiments in question (No. 126) did any of the test animals become infested, and this might be taken to indicate that the trichinae had suffered somewhat

because of the longer periods elapsing since the removal of the meat from the freezer, inasmuch as in other experiments in which the period of exposure in the freezer had been about the same but in which the meat was fed more promptly positive results were obtained in the feeding tests—i. e., in experiments 31, 122, 123, and 121, the periods elapsing between removal from the freezer and the first feeding of test animals being 2, 2, 2, and 3 days, respectively. This comparison, however, is not of great value, since in experiments 15, 28, 27 (meat in freezer at about 15° F. for 12 to 13 days), and 125 (meat in freezer at about 15° for 22 days) in which the meat was fed 2, 5, 5, and 4 days, respectively, after removal from the freezer, the results of the feeding tests were negative.

Further investigation is required to determine the changes which occur in the vitality of trichinæ when frozen meat is kept for varying periods of time after thawing. From the data at present available, however, it is quite certain that if any considerable changes occur, they are in the direction of a lowering of vitality and not in the reverse direction.

In this connection it is of interest to note that in unfrozen meat kept over three months after slaughter the trichinæ had suffered no evident loss in vitality, and small quantities of the meat were sufficient to produce heavy infestations in rats (controls, experiments 91 to 126). On the other hand, in meat kept nearly eight months after slaughter the trichinæ had lost their vitality, and test rats failed to become infested (controls, experiments 72 to 76).

#### EFFECTS OF ARTIFICIAL DIGESTION UPON TRICHINÆ

As evident from the tabular statement of the experiments (control examinations), artificial digestion for 24 hours or less had no appreciably injurious effect upon the vitality of trichinæ. When digested for two days, however, a considerable proportion of the trichinæ are liable to be killed (experiment 32). On the other hand, if 5 or 6 gm. of salt are added to each liter of digestive fluid the vitality of the trichinæ is not so seriously affected. The trichinæ from unfrozen meat digested for two days in experiment 96 seemed as lively as usual. Trichinæ, however, from meat frozen for 16 days at about 15° F. in experiment 120 evidently suffered considerably from digestion for two days, inasmuch as a smaller proportion were active and these were less lively than trichinæ examined in experiments 121, 122, 123, from meat frozen 17, 18, and 20 days, respectively, at about 15° F. and digested less than 24 hours. Furthermore, the fact that prolonged digestion in a digestive fluid containing 0.5 per cent of sodium chlorid is injurious to trichinæ from unfrozen meat was shown by an experiment in which digestion was continued for four days. In this instance all of the trichinæ were killed.

Though it is possible that the methods of artificial digestion employed in the experiments to free trichinæ from meat for examination reduced

their vitality so that many were found to be inactive which before digestion were still alive, the results of the examinations corresponded very well with the feeding tests. In fact, the examinations not uncommonly showed some of the trichinæ to be still alive, whereas in the corresponding feeding tests with the same meat not artificially digested none of the test animals became infested. On the other hand, there was no case in the freezing experiments in which the feeding test resulted in infection and the corresponding examination failed to reveal living trichinæ unless experiment 86 be taken as an exception. In this experiment, following a negative examination of digested meat, 3 larval trichinæ were found in the intestine of one of the test rats, which died four days after the first feeding; one of these larvæ was alive and exhibited feeble movements, but none of the 3 had undergone any development; the 4 other test rats failed to become infested. Experiment 67 was nearly an exception to the rule, as only 2 live trichinæ were found among 285 examined, the feeding test resulting positively. Only one out of four test rats became infested, however, and this one had but 3 trichinæ in the diaphragm. On the whole, the method of artificial digestion appears to afford a more rigorous test of the viability of trichinæ than the feeding of experimental animals in view of the fact that trichinæ are often found to be alive in digested meat when the feeding of the undigested meat to experimental animals fails to produce infection.

As a rule, in testing meat it is preferable not to depend alone upon the results of artificial digestion or the results of feeding test animals, but to employ both methods and take the results of both into consideration.

It is quite evident from the results of the experiments that artificial digestion is a valuable method for testing the viability of trichinæ, and that when properly controlled its injurious effects upon their vitality are so slight as to be practically negligible. The following formula may be recommended as fully satisfactory:

Water.....	1,000 c. c.
Hydrochloric acid (sp. gr. 1.19).....	10 c. c.
Scale pepsin (U. S. P.).....	2.5 gm.
Sodium chlorid.....	5 gm.

Fifty grams of ground meat are to be stirred into 600 c. c. of the digesting fluid, warmed to 38° or 40° C., and incubated for about 18 hours at this temperature.

#### LONGEVITY OF TRICHINÆ AFTER ARTIFICIAL DIGESTION

Trichinæ freed from their capsules by artificial digestion have been kept alive in tap water for 15 days. In one case 73 out of 75 were active at the end of this time. When examined again, 13 days later, all were dead. Kept in a 0.6 per cent sodium-chlorid solution for 16 days, 41 out of 43 examined were alive, some of them being sluggish but most of them

moderately lively. In another lot kept in a 0.6 per cent sodium-chlorid solution for 26 days, 15 out of 24 were alive and moderately active when warmed. Examined again 24 days later, all were dead. In a lot kept in 2 per cent sodium-chlorid solution for 11 days, 37 out of 38 were alive and very active. In these instances, after digestion of the meat, the trichinæ were washed in several changes of water or in physiological salt solution by decanting and settling. They were kept at ordinary room temperature. Numerous observations were made which showed that trichinæ freed from their capsules by artificial digestion will be apparently just as lively after several days if kept in water or physiological salt solution at ordinary room temperature as they are immediately after digestion.

If tap water containing trichinæ is kept at a temperature of 38° C. most of them are killed in a short time, but trichinæ may be kept an equal length of time at this temperature in a 0.6 per cent sodium-chlorid solution without apparent injury as shown by the following: Trichinæ from artificially digested meat were separated into two lots in beakers, one containing tap water, the other a 0.6 per cent sodium-chlorid solution. The two beakers were heated to 38° C. and this temperature maintained for 2½ hours. At the end of this time 23 out of 32 trichinæ from the tap water were inactive, whereas 18 examined from the salt solution were all active. The two beakers after replacing the tap water in one with a 0.6 per cent sodium-chlorid solution were kept at room temperature until the following day and then reexamined. Out of 108 trichinæ examined in the one case (heated in tap water), 81 were found to be inactive, whereas in the other case (heated in salt solution) all but 1 out of 100 examined were active.

On another occasion some trichinæ in tap water were kept at a temperature of 32° C. for about half an hour. Most of them became inactive but resumed their activity when the water was replaced with a 0.6 per cent sodium-chlorid solution, although their color became darker than normal and vacuoles appeared in the lateral fields.

It was on account of this discovery of the injurious effects of warm tap water that in the later experiments when meat was digested artificially it was washed with salt solution instead of tap water, and that salt solution instead of tap water was used as an examination medium. The use of tap water in the earlier experiments, however, probably affected the results of the examinations little, if any, as they are evidently quite consistent with the results of the later experiments (see Tables I and II). The washing was done with cold tap water, and in examining the trichinæ they were transferred a few at a time to a warm stage, where they were kept only a few minutes, too short a period for the injurious effects of immersion in warm water to become established, as was repeatedly demonstrated in using this method upon trichinæ from unfrozen meat.



## TEST ANIMALS

It will be noted from Table I that of the 54 test animals (53 rats, 1 guinea pig) fed with unfrozen meat as controls upon the animals fed with frozen meat, only 3 failed to become infested. The rats fed as controls in experiments 72 to 76 are left out of consideration, as they were not fed until nearly eight months after the slaughter of the hog from which the meat was taken. Examination of some of the meat artificially digested nine months after slaughter of the hog showed that the trichinae were dead. One out of three rats fed as controls in experiments 23 to 34 showed no infection, the two others being heavily infested. Out of 29 rats fed as controls in experiments 65, 65a, and 67 to 71, 1 showed no infection, 27 of the remaining 28 showing heavy infections. Finally, 1 out of 12 rats fed as controls in experiments 91 to 126 showed no infection, but this one was killed four days after feeding for another purpose and as only a small portion of the intestine was examined, trichinae may have been present and were not discovered; 8 of the remaining rats were heavily infested; in the case of the 3 others the degree of infestation was not recorded.

These results, particularly in view of the fact that the control animals as a rule received much smaller quantities of meat than those fed on meat which had been frozen, demonstrate the adequacy of the methods employed in feeding test animals. The results of the later experiments, however, beginning with No. 23 are considered more reliable, so far as the feeding tests are concerned, than those of the earlier experiments, as more animals were used and care was taken to feed larger quantities of meat. The method of feeding each lot of test rats together in a cage a certain amount of meat on several successive days, followed in most of the experiments, appeared to be quite satisfactory. Undoubtedly some of the rats in each lot ate more of the meat than others, so that some inequality in the degree of infestation of the rats would be likely, which, however, was of little importance, as the results of the feeding tests were judged upon the basis of the findings in all of the rats in each lot. The use of a number of rats for each test allowed larger quantities of meat to be tested, which gives a decided advantage over the use of a single animal. For the same reason, rats are preferable to guinea pigs, as they will eat of their own accord much larger quantities of meat than can readily be fed to guinea pigs forcibly or by mixing with lettuce, cabbage, etc. Furthermore, it is difficult to induce guinea pigs to eat chopped meat mixed with lettuce or other materials if the meat has become only slightly tainted, whereas rats usually eat meat readily even after it has become very stale or partially decomposed.

## SUMMARY AND CONCLUSIONS

Prior to the investigations recorded in the present paper very little experimental work had been done upon the effects of cold upon encysted trichinae, and the current belief was that low temperatures do not seriously affect the vitality of these parasites. This belief is shown to have been erroneous by the results of numerous experiments.

Quantities of trichinous meat varying in weight from a few grams to nearly 400 pounds were frozen and kept for periods varying from a few minutes to 57 days at various temperatures below the freezing point of water. Usually the process of refrigeration was carried out in cold-storage compartments known as freezers, but in a few cases in which the low temperature was maintained only a short time, a freezing mixture was employed. In most cases the period of refrigeration was between 5 and 20 days. The meat on removal from the freezer was generally allowed to thaw slowly at ordinary house temperatures; in a few cases, in order to study the effects of rapid thawing, the process was hastened by breaking apart the pieces of frozen meat so that they thawed completely in a few minutes. Generally the meat after thawing was treated as follows: A portion was chopped or ground into fine pieces, placed in an artificial gastric juice, and incubated at 38° to 40° C. overnight, and then washed with water or a physiological salt solution by decanting and sedimenting. The sediment containing the trichinae isolated from their capsules was examined microscopically on a warm stage, and the number of inactive and active ones recorded, together with such other observations as appeared worthy of remark. For the purpose of controlling the effects of the process of digestion, some trichinous meat, nearly always from the same carcass as the frozen meat, which had been kept in an unfrozen condition, was digested at the same time, using some of the same lot of digesting fluid. Another portion of the frozen meat after thawing was fed to test animals, in most cases to white or hooded rats specially reared to avoid chances of accidental infection; as a rule, five rats were fed, receiving the meat on several successive days. Finally, unfrozen meat from the same carcass as that used in a given refrigeration experiment was fed to control test animals, usually in much smaller quantities than in the case of the frozen meat. In some instances no control test animals were fed. The test animals as they died, or after about a month if they lived that long, were examined for trichinae, the intestines as well as the diaphragm being examined if they died within the first two weeks after feeding; otherwise only the diaphragm. About 30,000 trichinae were examined from artificially digested frozen and unfrozen meat, and over 500 test animals and control animals were fed and examined.

A considerable proportion of the trichinae in meat exposed to a temperature of about 15° F. for periods of 23 days or less survive and are

quite lively after thawing, but such meat frequently fails to infect test animals. This temperature is injurious to trichinæ, but its effects are uncertain, and meat exposed as long as 23 days has proved to be infectious. Some of the trichinæ in meat exposed to a temperature of about  $10^{\circ}$  for periods of 57 days or less generally survive, but the meat frequently fails to infect test animals. A temperature of  $10^{\circ}$  is more injurious to trichinæ than a temperature of  $15^{\circ}$ , but, like the latter, its effects are uncertain, although meat exposed to it for 14 days or longer has generally failed to produce infestation; or if infestation resulted it was slight. No infestation has been produced by trichinous meat exposed to a temperature of about  $10^{\circ}$  for 20 days or longer.

Apparently in the neighborhood of  $10^{\circ}$  F. a critical point is reached below which the effects of cold upon trichinæ become suddenly much more pronounced.

Temperatures of  $5^{\circ}$  F. or lower profoundly affect the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are very unlikely to result. Slight infections, however, have resulted from meat exposed to a temperature of  $-9^{\circ}$  to  $0^{\circ}$  for 10 days.

Trichinæ from meat exposed to temperatures below  $15^{\circ}$  F. when examined microscopically after thawing exhibit changes in the appearance of the protoplasm. A temperature of  $10^{\circ}$  produces greater changes than  $15^{\circ}$ , and the changes produced by a temperature of  $5^{\circ}$  are still more pronounced. The more conspicuous of these changes are more or less loss of color of the esophageal cell body, more or less solidification of its nuclei, abnormal granulation of its protoplasm, and more or less dissociation and rounding of the germ cells.

Trichinæ vary in their resistance to cold, and some individuals survive refrigeration longer and at lower temperatures than others.

Within certain limits the rapidity with which trichinous meat freezes or thaws has no appreciable effect upon trichinæ. Apparently the rapid freezing and thawing undergone by very small pieces of meat (a few grams in weight) adds to the injurious effects of refrigeration, but the natural variations in the rate of freezing and thawing dependent upon variations in the quantities of meat frozen between limits of half a pound and several hundred pounds do not noticeably modify the effects of refrigeration upon trichinæ.

The vitality of trichinæ which survive refrigeration does not decrease noticeably during a period of at least a week after the thawing of the meat.

The artificial digestion of trichinous meat for 24 hours at a temperature of  $38^{\circ}$  to  $40^{\circ}$  C. in a fluid consisting of water, 1,000 c. c., hydrochloric acid (sp. gr. 1.19), 10 c. c., scale pepsin (U. S. P.), 2.5 gm., and sodium chlorid, 5 gm., has no apparent effect upon the activity or structure of the trichinæ, released from their capsules by the process of digestion.

Trichinæ thus released from their capsules may remain alive and retain their normal activity for 10 days or more when kept in a 0.6 per cent sodium-chlorid solution at a temperature of about 20° C., and have been found alive and moderately active at the end of 26 days. They may likewise be kept alive for two weeks or more in tap water at a temperature of about 20° C. Trichinæ have been kept alive and very active for 11 days in a 2 per cent sodium-chlorid solution at a temperature of about 20° C. Trichinæ in tap water warmed to a temperature of 38° C. become inactive within a few hours, but may be kept in a 0.6 per cent sodium-chlorid solution at this temperature for a similar length of time without apparent injury.

In the practical application of refrigeration as a means of destroying the vitality of trichinæ, meat should be refrigerated at a temperature not higher than 5° F. for not less than 20 days, a period which allows a probable margin of safety of nearly 10 days. The employment of higher temperatures of refrigeration as a means of destroying the vitality of trichinæ is not justified in the light of our present knowledge because of the uncertainty of the effects of such temperatures. Whether temperatures higher than 5° F. may be safely employed by lengthening the period of refrigeration remains to be determined.

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# RELATION BETWEEN CERTAIN BACTERIAL ACTIVITIES IN SOILS AND THEIR CROP-PRODUCING POWER

By PERCY EDGAR BROWN,

*Chief in Soil Chemistry and Bacteriology, Iowa Agricultural Experiment Station*

## INTRODUCTION

Soil-bacteriological investigations in the past have dealt almost exclusively with the occurrence and activities of micro-organisms in the soil, and no attempt has been made, from the standpoint of crop production, to interpret the results obtained.

A knowledge of the relation of soil bacteria to soil fertility is of considerable importance, however, if the subject is to be of any value in practical agriculture. While, therefore, much work on methods remains to be done, so much knowledge concerning bacterial action in soils has been accumulated during the last few years that it seems time now to call attention to the practical phase of the subject, to attempt at least to correlate the results secured with known facts regarding soil fertility.

The purpose of these experiments has been to study certain bacterial activities in field soils in the attempt to secure information regarding their relation to the actual crops produced. If special methods of soil treatment exert similar effects on certain bacterial activities and on crops, it may be assumed that there is a fairly definite relation between the two, and the particular bacterial activities in a soil may indicate its crop-producing power. Thus, if in laboratory tests the ammonifying power, the nitrifying power, or the azofying power of a soil is enhanced by some method of soil treatment and the crop production is also increased, the conclusion that ammonification, nitrification, or azofication and crop production are very closely related would be well warranted. Tests of such bacterial action in soils would therefore constitute a means of ascertaining their crop-producing power, and the importance of obtaining advance information along this line is evident.

Experiments covering many years of varying seasons and including tests of all varieties of treatments must, of course, be carried out before any definite conclusions can be reached. The experiments reported here were secured on three series of plots under definite systems of treatment, and it was intended in undertaking the work to carry it on for a long period of years before attempting to draw conclusions. Inasmuch, however, as the particular plots were of necessity relinquished, owing to the development of certain departments of the State College, and studies of a like nature can not be undertaken on new plots until several years of special treatment have elapsed, it has been deemed

expedient to assemble the data obtained up to the present time and to offer them as a preliminary contribution along this line. The fact that many of the data are rather positive in nature has been an added reason for presenting them at this time. Portions of the results have been published in other connections, while others have not previously been reported, but in either case average results only are included here.

#### FIELD SOILS STUDIED

Three series of field plots have been used in this work, one consisting of 14 plots one-tenth of an acre in size, located on a uniform soil in the Wisconsin drift-soil area, and classed by the United States Bureau of Soils as Carrington loam.

Prior to 1907 it had been under a regular 4-year rotation and had been subjected to no special treatment of any kind. In that year the plots were differentiated according to the following plan:

Plot No.	Treatment.
601 . . . . .	Continuous corn.
602 } . . . . .	2-year rotation. Corn and oats.
603 }	
604 } . . . . .	3-year rotation. Corn, oats, and clover.
605 }	
606 }	
607 } . . . . .	2-year rotation. Corn and oats, clover plowed under after the oats.
608 }	
609 } . . . . .	2-year rotation. Corn and oats, cowpeas plowed under after the oats.
610 }	
901 } . . . . .	2-year rotation. Corn and oats, rye plowed under after the oats.
902 }	
903 . . . . .	Continuous clover.
904 . . . . .	4-year rotation. Corn, oats, and clover.

The first tests of these soils were carried out in 1911, the fourth year of the special treatment. Results were secured also in 1912 and 1913, only a few data being obtained in the latter year owing to the pressure of other work, but the ammonification studies were complete. During each season only those plots under corn were examined, as the effects of previous treatment could, of course, hardly be studied on plots under different crops, and furthermore it would be evidently impossible to compare the crop yields on the various plots if the same crop were not grown. Different plots in this series were thus examined in the different years, but in each case the same treatments were included in the study.

The second series of plots consisted of 5 one-tenth-acre plots on the same soil area and on the same soil types as the previous series. In the fall of 1910 these plots were subjected to the special treatments indicated below:

Plot No.	Treatment.
1004 . . . . .	Check.
1005 . . . . .	8 tons of manure per acre.
1006 . . . . .	12 tons of manure per acre.
1007 . . . . .	16 tons of manure per acre.
1008 . . . . .	20 tons of manure per acre.

The study of these plots was carried out in 1912, the crop grown that year being corn.

The third series of plots was composed of 3 one-twentieth-acre plots located on the same soil type as the other series.

Special treatment on these soils consisted in the application of lime as follows:

	Plot No.	Treatment
510 . . . . .		Check.
509 . . . . .		2 tons of ground limestone per acre.
508 . . . . .		3 tons of ground limestone per acre.

The lime was applied to these plots just prior to the corn planting, and the tests of the soils were carried out later in the same season.

#### BACTERIOLOGICAL METHODS

The solution method for testing bacterial activities in soils has been studied in some detail by several investigators, and, while results of much value have been secured by its use, there are certain difficulties attendant upon it which have not yet been obviated. These difficulties have been discussed in another publication<sup>1</sup> and need not be entered upon here. The use of soil itself as a medium for studying bacterial activities in field soils seems at the present time the most logical method. Modified solutions such as have been suggested in recent work<sup>2</sup> can hardly be considered as satisfactory as soil itself in representing the physical and chemical conditions in field soils, leaving out of account entirely the bacteriological factor.

The addition of various materials to soils in laboratory tests to permit the accumulation of the particular products of bacterial action which it is desired to measure has been studied. Dried blood, cottonseed meal, and casein have proved the best for ammonification; dried blood and ammonium sulphate for nitrification; and mannite for azofication.

In this work various modifications of the soil method were employed for the reason that the tests were carried out during a period of several years through which experiments on methods were also being conducted. The results, using the different methods, are all included, however, as they all tend in the same direction, and conclusions are based on a study of the entire mass of data secured.

#### EXPERIMENTAL WORK

##### TESTS ON ROTATION PLOTS IN 1911

Four samplings were made during 1911—on June 26, July 8, September 16, and October 25—and tests made of the soils for their ammonifying, nitrifying, and azofying powers. The yield of corn was secured from the plots examined.

<sup>1</sup> Brown, P. E. Methods for bacteriological examination of soils. Media for quantitative determination of bacteria in soils. Iowa Agr. Exp. Sta. Research Bul. 11, p. 379-407. 1913.

<sup>2</sup> Löhnis, Felix, and Green, H. H. Methods in soil bacteriology. VII. Ammonification and nitrification in soil and solution. *Z. Centbl. Bakt. [etc.]*, Abt. 2, Bd. 40, No. 19/21, p. 457-479. 1914.



Complete data obtained in this work have been given in another place,<sup>1</sup> and hence only summarized results are included here.

The results of the ammonification tests with dried blood and cottonseed meal are given in Tables I and II, respectively. The nitrification tests with ammonium sulphate and dried blood appear in Tables III and IV, and the azofication results are given in Table V.

TABLE I.—*Ammonification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601. . . . .	171. 11	220. 74	108. 76	110. 58
602. . . . .	178. 07	231. 38	117. 86	116. 54
604. . . . .	188. 82	243. 60	133. 43	131. 11
607. . . . .	175. 22	229. 63	129. 78	124. 82
609. . . . .	179. 96	238. 53	118. 53	116. 84
901. . . . .	174. 75	232. 08	117. 04	114. 88

TABLE II.—*Ammonification of cottonseed meal on rotation plots in 1911*

Plot No	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601. . . . .	142. 01	163. 32	102. 13	111. 08
602. . . . .	144. 54	168. 74	110. 09	122. 17
604. . . . .	151. 18	177. 81	120. 18	126. 64
607. . . . .	145. 49	168. 21	131. 11	123. 49
609. . . . .	148. 50	171. 00	105. 78	119. 02
901. . . . .	144. 07	165. 94	112. 73	115. 55

TABLE III.—*Nitrification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601. . . . .	12. 442	19. 883	11. 864	13. 797
602. . . . .	15. 196	23. 311	14. 629	17. 433
604. . . . .	20. 776	27. 087	18. 173	24. 032
607. . . . .	15. 078	22. 884	16. 410	22. 211
609. . . . .	18. 798	25. 226	13. 453	15. 048
901. . . . .	13. 962	20. 713	12. 711	14. 014

<sup>1</sup> Brown, P. E. Bacteriological studies of field soils. II. The effects of continuous cropping and various rotations. Iowa Agr. Exp. Sta. Research Bul. 6, p. 211-246. 1912.

TABLE IV.—*Nitrification of ammonium sulphate on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601. ....	5. 019	17. 577	7. 565	8. 086
602. ....	8. 075	21. 625	9. 788	11. 789
604. ....	12. 630	24. 517	12. 903	19. 419
607. ....	7. 066	21. 477	11. 357	13. 749
609. ....	11. 908	22. 978	9. 101	10. 620
901. ....	6. 724	21. 477	8. 310	9. 655

TABLE V.—*Azofication tests on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601. ....	9. 50	3. 93	13. 52	10. 32
602. ....	17. 46	15. 07	19. 92	17. 52
604. ....	20. 64	18. 25	23. 12	20. 72
607. ....	14. 27	17. 46	20. 72	18. 32
609. ....	18. 25	15. 87	18. 32	16. 72
901. ....	14. 27	11. 88	16. 72	15. 12

The variations in the amount of moisture in the different plots at the same samplings were very small and the differences in bacterial activities which were found could not, therefore, be attributed to the different moisture conditions in the plots.

The yields obtained with corn on the various soils are given in Table VI, and comparing these with the ammonification, nitrification, and azofication results it will be noted that there is a remarkably good agreement.

TABLE VI.—*Yield of corn on rotation plots in 1911*

Plot No.	Treatment	Yield per acre
		<i>Bu.</i>
601	Continuous corn .....	35. 5
602	2-year rotation .....	46. 0
604	3-year rotation .....	50. 7
607	2-year rotation; clover turned under .....	52. 7
609	2-year rotation; cowpeas turned under .....	32. 5
901	2-year rotation; rye turned under .....	43. 2

The ammonification results with the dried blood and cottonseed meal did not always run exactly parallel, but the differences were slight, and in most cases the same comparisons were secured, so they need not be

considered separately. The same is true of the nitrification results with ammonium sulphate and dried blood.

Furthermore, the ammonification, nitrification, and azofication results are all in close agreement as to the relative effects on each of the various treatments; and, hence, the bacteriological results may be compared as a whole with the crop yields.

An examination of the tables reveals the fact that a greater crop yield was secured where the 2-year rotation was followed than on the continuous corn plot, and a still greater yield was secured where the 3-year rotation was followed. Exactly the same relations were found in the ammonification, nitrification, and azofication results.

Where the clover was introduced into the 2-year rotation as a green manure a greater crop yield was obtained than where it was not used. Furthermore, a slightly greater yield was obtained than on the 3-year rotation plot. The bacteriological results are not in accord with these differences; but in most cases the variations were not large, and the differences in crop yield were not great. Hence, the lack of agreement here should not be considered of great significance.

When cowpeas were used in the 2-year rotation, however, the yield was abnormally depressed. The bacterial activities were also depressed, but not to so great an extent. Evidently some unknown factor interfered here, as such a depression is hardly explainable. Where rye was turned under in the 2-year rotation the yield was less than on the regular 2-year rotation plot, and corresponding depressions were noted in the bacterial activities.

It is apparent that the ammonification, nitrification, and azofication results as a whole show a surprisingly close relation to the crop yield. Nitrification and ammonification tests frequently proceed in the same direction, and it is possible that after many confirmatory tests have been carried out it may be found that only one of these bacteriological tests of soils needs to be made. At the present time, however, the data available along this line are insufficient to warrant the interpretation of the results from one process as fitting another.

It is hardly expected, however, that azofication results will run parallel with ammonification and nitrification tests in any large number of studies. Conditions which favor the latter processes need not necessarily favor azofication.

These results as a whole, therefore, indicate that under normal soil conditions the ammonifying and nitrifying powers of soils may reflect fairly accurately their crop-producing power and show quite accurately the relative yields which will be secured. Only in special cases can similar dependence be placed on azofication results. These tentative conclusions have been further tested and are borne out by the later results.

## TESTS ON ROTATION PLOTS IN 1912

The same series of plots was used in 1912 in the study of the relative effects of different rotations on bacterial activities and on crop production, but in some cases different individual plots were employed, as again only those which were cropped to corn were examined.

Ammonification tests were carried out by the dried-blood-air-dry-soil method with inoculum from fresh soil, the casein-fresh-soil method, and the dried-blood-fresh-soil method. The nitrifying power was tested by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. These methods were under investigation at the time of this study, and comparative tests of their efficiency have been reported in the work already referred to.<sup>1</sup>

Four samplings were made during the year—on August 9, August 19, October 7, and October 26. The variations in moisture content of the soils at the various dates were so slight that the differences observed could not be attributed to that factor, and the results of the determinations are not included here.

The crop yields were obtained from the plots as in the previous year.

The ammonification results appear in Tables VII, VIII, and IX, the nitrification results in Tables X and XI, and the crop yields are given in Table XII.

TABLE VII.—*Ammonification of dried blood in air-dry soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen)			
	Test 1.	Test 2.	Test 3.	Test 4.
601.....	148. 33	54. 93	124. 78	122. 42
603.....	157. 55	66. 31	130. 27	127. 92
605.....	170. 09	79. 77	138. 71	138. 71
608.....	172. 65	82. 40	141. 85	143. 42
610.....	168. 53	75. 73	136. 95	130. 67
902.....	151. 27	64. 15	125. 17	119. 09
904.....	161. 08	71. 61	131. 06	138. 21

TABLE VIII.—*Ammonification of dried blood in fresh soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen)			
	Test 1.	Test 2.	Test 3.	Test 4.
601.....	106. 34	68. 66	50. 81	54. 74
603.....	110. 66	80. 05	65. 14	62. 39
605.....	117. 32	86. 70	73. 77	71. 02
608.....	120. 87	88. 28	74. 02	74. 66
610.....	115. 95	78. 87	72. 59	71. 41
902.....	109. 67	73. 38	58. 86	62. 19
904.....	114. 14	82. 90	68. 28	69. 17

<sup>1</sup> Brown, P. E. Op. cit.

TABLE IX.—*Ammonification of casein on rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601.....	61. 80	64. 84	58. 66	55. 33
603.....	67. 30	71. 80	65. 13	66. 31
605.....	71. 61	76. 52	68. 47	69. 45
608.....	72. 39	79. 07	70. 63	72. 79
610.....	68. 67	73. 37	67. 29	69. 25
902.....	62. 78	69. 06	63. 18	62. 39
904.....	67. 10	73. 37	67. 10	68. 67

TABLE X.—*Nitrification of ammonium sulphate in the air-dry soil of rotation plots in 1912*

Plot No.	Quantity of nitrates (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601.....	10. 431	12. 443	8. 444	7. 232
603.....	13. 489	16. 751	12. 427	11. 333
605.....	15. 114	18. 941	15. 546	14. 557
608.....	15. 250	23. 931	16. 524	15. 250
610.....	14. 196	18. 110	15. 208	14. 733
902.....	12. 695	12. 893	9. 914	10. 936
904.....	14. 434	17. 410	14. 946	14. 686

TABLE XI.—*Nitrification of ammonium sulphate in the fresh soil of rotation plots in 1912*

Plot No	Quantity of nitrates (in milligrams of nitrogen)			
	Test 1	Test 2	Test 3.	Test 4.
601.....	11. 944	15. 300	7. 183	6. 844
603.....	12. 728	16. 601	10. 695	9. 776
605.....	14. 682	22. 583	12. 462	12. 154
608.....	15. 520	25. 078	13. 784	14. 224
610.....	13. 559	18. 264	12. 233	13. 999
902.....	11. 960	15. 837	7. 789	10. 629
904.....	13. 060	17. 414	10. 981	13. 166

TABLE XII.—*The yield of corn on rotation plots in 1912*

Plot No.	Treatment.	Yield per acre.
601	Continuous corn .....	Bu. 50. 25
603	Corn and oats .....	63. 12
605	Corn, oats, and clover .....	69. 00
608	Corn and oats; clover turned under .....	74. 00
610	Corn and oats; cowpeas turned under .....	68. 50
902	Corn and oats; rye turned under .....	59. 50
904	Corn, corn, oats, and clover .....	67. 50

If these results are examined, it is found that practically uniform agreement was secured with the various methods—i. e., the relative ammonifying powers of the soils were the same whether the dried-blood or the casein method was employed, and it made little difference whether the dried-blood-air-dry-soil method was employed or the dried-blood-fresh-soil method was used. Similarly, in the case of nitrification, the same relative results were obtained whether the air-dry-soil method or the fresh-soil method was employed. It is unnecessary, therefore, to consider the results individually, and comparisons will merely be made between the bacterial results and the crop yields.

The largest crop yield was obtained in this year on the plot under the 2-year rotation with clover turned under. Similarly, the greatest ammonifying power and the greatest nitrifying power were found in this soil. The soil under the 3-year rotation (corn, oats, and clover) was second in crop yield and in bacterial activities; the 2-year rotation with cowpeas as a green manure induced a slightly smaller crop yield and lower bacterial action; the 4-year rotation was still lower; the 2-year rotation (corn and oats) lower yet; the 2-year rotation with rye turned under gave a still smaller crop yield and lower bacterial action; and the continuous-crop plot was at the bottom of the list.

It is evident from these results that the ammonification and nitrification of nitrogenous organic material in soils and their crop-producing power are very closely related and that tests of the power of soils to produce ammonia or nitrates may be an indication of their crop-producing power, or at least of their relative crop-producing ability. Previous results are also confirmed regarding the similarity of the effects of soil treatment or ammonification and nitrification. Such need not always be the case, of course, as it is possible to conceive of conditions affecting the nitrifying organisms which do not similarly affect the ammonifiers, but it seems to be the case that in ordinary field conditions the two processes are quite similarly affected by treatment and probably only one process need be tested to gain some idea of the relative crop-producing power of soils.

#### TESTS ON ROTATION PLOTS IN 1913

The experiment on the same series of plots was continued in 1913, different individual plots being used for corn.

Three samplings were made during the season—on August 15, August 23, and August 26. Ammonification tests only were carried on, owing to the pressure of other work; and only one method, the casein-fresh-soil method, was employed. The crop yields were obtained as previously. Again, the moisture content of the soils at the different samplings varied so slightly that the differences may be considered negligible from the standpoint of the effects of treatment.

The results of the ammonification tests appear in Table XIII, and the crop yields are given in Table XIV.

TABLE XIII.—Ammonification of casein on rotation plots in 1913

Plot No.	Quantity of nitrogen.		
	Aug. 15.	Aug. 23.	Aug. 26.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
601.....	68.38	60.82	55.67
602.....	71.56	63.47	59.31
606.....	78.74	69.59	64.05
607.....	74.89	66.35	63.15
609.....	73.53	64.45	60.52
901.....	75.65	68.15	63.46
904.....	74.28	65.21	60.97

TABLE XIV.—Yields of corn on rotation plots in 1913

Plot No.	Treatment.	Yield per acre.
		<i>Bu.</i>
601	Continuous corn.....	30.0
602	2-year rotation: Corn and oats.....	53.3
606	3-year rotation: Corn, oats, and clover.....	68.0
607	2-year rotation: Corn and oats; clover turned under.....	64.0
609	2-year rotation: Corn and oats; cowpeas turned under.....	60.0
901	2-year rotation: Corn and oats; rye turned under.....	65.3
904	4-year rotation: Corn, corn, oats, and clover.....	62.6

Comparing the results, it is apparent that the indications of fertility given by the ammonification studies were borne out by the actual crop yields. The rank of the soils both in ammonifying power and in crop production was as follows:

Plot No.	Treatment.	Rank.
606	3-year rotation.....	1
901	2-year rotation; rye turned under.....	2
607	2-year rotation; clover turned under.....	3
904	4-year rotation.....	4
609	2-year rotation; cowpeas turned under.....	5
602	2-year rotation.....	6
601	Continuous corn.....	7

The results of these studies check those of previous years, therefore, and indicate that ammonification and crop production are very closely related and that the determinations of the ammonifying power of soils made during the growing season may show their relative crop-producing powers.

The plots in this series, as will be noted, ranked differently each year, both in crop yields and in bacterial activities, but it is not purposed to enter here upon a discussion of the reasons for such variations. The seasonal conditions, especially as regards rainfall, were undoubtedly of

prime importance. It will be noted, however, that the rotation of crops increased in every case both the crop yield and the bacterial activities. The use of green manure in the 2-year rotation sometimes proved more valuable than the 3-year rotation, and sometimes was of less value. This was probably due also to the moisture conditions. The point of importance here is, however, the fact that, regardless of seasonal conditions or of the effect on crops under particular conditions, bacterial activities and crop production were relatively similar.

#### TESTS ON MANURED PLOTS IN 1912

The manured plots were studied in 1912. Ammonification results were obtained by the casein-fresh-soil method, the dried-blood-air-dry-soil method, and the dried-blood-fresh-soil method; and nitrification tests were carried out by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. Four samplings were made during the season—on August 2, August 15, August 22, and September 9. The moisture conditions in the soils varied so slightly that they could not be considered of significance, and they are not included here. Crop yields were secured, corn being grown on the plots as in the other series.

Complete data from these experiments have been reported in another place<sup>1</sup> and only summarized results are given here.

The ammonification results are given in Tables XV, XVI, and XVII, the nitrification results in Tables XVIII and XIX, and the crop yields in Table XX.

TABLE XV.—*Ammonification of dried blood in the fresh soil of manured plots in 1912*

Plot No	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
1004.....	66. 90	83. 97	73. 57	66. 71
1005.....	84. 76	92. 21	83. 97	70. 63
1006.....	86. 32	106. 34	98. 88	85. 54
1007.....	97. 90	109. 47	98. 88	84. 95
1008.....	86. 72	95. 74	87. 50	76. 91

TABLE XVI.—*Ammonification of casein on manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
1004.....	37. 87	68. 27	67. 49	51. 60
1005.....	46. 89	73. 57	72. 79	58. 86
1006.....	51. 79	77. 50	78. 87	66. 32
1007.....	51. 99	78. 48	79. 46	65. 72
1008.....	48. 78	75. 14	74. 75	60. 42

<sup>1</sup>Brown, P. E. Bacteriological studies of field soils. III. The effects of barnyard manure. Iowa Agr. Exp. Sta. Research Bul. 13, p. 421-448. 1913.



TABLE XVII.—*Ammonification of dried blood in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
1004.....	80. 44	111. 83	106. 34	102. 81
1005.....	94. 76	117. 33	109. 47	117. 13
1006.....	100. 06	131. 25	122. 23	127. 92
1007.....	100. 85	137. 14	124. 00	133. 02
1008.....	95. 75	128. 90	113. 80	122. 62

TABLE XVIII.—*Nitrification of ammonium sulphate in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
1004.....	8. 507	14. 794	12. 500	9. 211
1005.....	9. 326	15. 453	13. 693	10. 262
1006.....	10. 000	17. 710	14. 392	12. 593
1007.....	11. 655	18. 712	16. 401	12. 446
1008.....	10. 064	16. 696	14. 662	10. 444

TABLE XIX.—*Nitrification of ammonium sulphate in the fresh soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
1004.....	5. 576	10. 946	10. 283	9. 141
1005.....	7. 259	12. 583	12. 543	10. 000
1006.....	8. 470	16. 733	14. 142	12. 698
1007.....	10. 282	18. 694	15. 641	13. 011
1008.....	8. 125	16. 164	12. 949	10. 528

TABLE XX.—*Yield of corn on manured plots in 1912*

Plot No.	Treatment.	Yield per acre.
		<i>Bu.</i>
1004	Check.....	50. 50
1005	8 tons of manure.....	77. 62
1006	12 tons of manure.....	86. 00
1007	16 tons of manure.....	87. 00
1008	20 tons of manure.....	81. 00

If the results secured in the ammonification tests are examined, it is seen that the effects of the manure were the same whatever method was employed. It is unnecessary, therefore, to consider the different results individually. Similarly in the case of nitrification, the fresh-soil and air-dry-soil methods yielded similar results, and general conclusions only need be drawn.

If the bacterial results as a whole are compared with the crop yields, it is found that there was exact agreement. Applications of manure increased the ammonifying and nitrifying powers of the soil, and the crop yield was also increased. Further gains in bacterial action and also in crop yields were obtained as the amount of manure applied was increased, but the maximum effect was obtained with the use of 16 tons of manure per acre. Beyond that point increasing the quantity of manure decreased both bacterial action and crop yields.

These results therefore check the previous observations that ammonification and nitrification tests may often run parallel. Previous results are also confirmed regarding the relation between crop yields and certain bacterial activities. Tests of the ammonifying power of soils or of their nitrifying powers apparently indicate quite accurately their crop-producing powers.

#### TESTS ON LIMED PLOTS IN 1911

The three plots in this series were sampled during 1911 on June 21, July 6, September 14, and October 24. Ammonification tests were made by the dried-blood and cottonseed-meal methods, nitrification by the ammonium-sulphate and dried-blood methods, and azofication by the mannite method. Crop yields were secured as in the other series studied. Complete results of these tests have been reported,<sup>1</sup> and only average results are given here.

The ammonification results appear in Tables XXI and XXII, the nitrification results in Tables XXIII and XXIV, the azofication results in Table XXV, and the crop yields in Table XXVI.

TABLE XXI.—*Ammonification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	Mgm.	Mgm.	Mgm.
510.....	207. 17	206. 60	128. 06	129. 78
509.....	208. 12	207. 30	144. 51	140. 05
508.....	214. 13	235. 22	155. 59	149. 32

<sup>1</sup> Brown, P. E. Bacteriological studies of field soils. I. The effects of lime. Iowa Agr. Exp. Sta. Research Bul. 5, p. 187-210. 1912.

TABLE XXII.—*Ammonification of cottonseed meal on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
510.....	131. 26	157. 22	126. 22	124. 32
509.....	132. 68	161. 06	141. 15	130. 28
508.....	142. 01	172. 58	151. 22	137. 90

TABLE XXIII.—*Nitrification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
510.....	13. 745	27. 056	20. 579	14. 570
509.....	15. 844	33. 857	23. 247	18. 434
508.....	21. 911	39. 686	29. 376	22. 946

TABLE XXIV.—*Nitrification of ammonium sulphate on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
510.....	8. 737	24. 987	14. 298	8. 762
509.....	10. 547	25. 475	20. 146	11. 743
508.....	14. 822	29. 034	24. 061	17. 890

TABLE XXV.—*Azofication tests on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
510.....	5. 52	2. 34	11. 89	11. 09
509.....	15. 07	16. 66	25. 41	27. 00
508.....	26. 21	30. 19	38. 93	37. 34

TABLE XXVI.—*Yield of corn on limed plots in 1911*

Plot No.	Treatment.	Yield per acre.
		<i>Bu.</i>
510	Check .....	52. 5
509	2 tons of lime .....	55. 0
508	3 tons of lime .....	55. 0

The ammonification results by the two methods employed were very similar, as also were the nitrification results; hence, these results need not be considered separately.

If the bacterial tests are compared with the crop yields, it is found that the lime increased ammonification, nitrification, and azofication in the soils, and the crop yield was similarly increased, the larger amount of lime bringing about the greater effect on the bacteria but exerting no further increasing effect on the crop grown.

These results as a whole therefore check those obtained on the plots under other methods of treatment and show that bacterial transformations of nitrogenous compounds in the soil or, rather, the ability of soils to bring about the simplification of nitrogenous materials or the addition of nitrogen, may be considerably modified by various methods of soil treatment. Furthermore, they check previous results in showing that certain bacterial activities in the soil may be very closely related to the actual crop-producing power of the soil. The ammonifying power of soils, their nitrifying power, or even, in certain cases, their azofying power may therefore indicate the crop-producing power of soils or, at least, their relative crop-producing power.

#### CONCLUSIONS

(1) These experiments as a whole represent a line of investigation in soil bacteriology which it is believed will ultimately place the subject on a more practical basis—a basis which will permit the direct application of the results obtained to the solution of soil-fertility problems.

(2) The relations between the bacterial activities studied and the actual crop yields on these plots have proved so striking and so consistent that it was felt that accidental coincidence had been practically eliminated and the results might be considered to give a strong indication that certain bacterial activities in field soils are very closely associated with crop yields.

(3) Furthermore, the tentative conclusion presents itself that tests of such bacterial activities in the laboratory may indicate quite accurately the crop-producing power of a soil or, at least, the relative crop-producing power of several soils.

(4) If, further, more exhaustive tests confirm these preliminary observations, it may be possible to secure advance information regarding the crop-producing power of soils by means of laboratory tests of bacterial action in those soils.

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## AGGLUTINATION TEST AS A MEANS OF STUDYING THE PRESENCE OF BACTERIUM ABORTUS IN MILK

By L. H. COOLEIDGE,

*Research Assistant in Bacteriology, Michigan Agricultural Experiment Station*

### INTRODUCTION

In the investigation of the effect on milk of the diseases of the cow, with special reference to infectious abortion, it was found desirable to examine a large number of samples to determine whether or not *Bacterium abortus* Bang was being passed with the milk. The cultural and animal-inoculation methods were the only ones found available for this work.

The cultural method devised by Nowak<sup>1</sup> takes advantage of the fact that newly isolated cultures require an atmosphere partially depleted of oxygen. This atmospheric condition is obtained by growing the agar streaks from suspected material in a closed jar with *Bacillus subtilis*, having 1 sq. cm. of culture surface to each 15 c. c. of jar capacity. While the author has isolated *Bact. abortus* from milk sediment by this method, it is too tedious a process to apply to any number of samples. Plates are likely to be overgrown with colonies of fast-growing organisms, and the method has the further disadvantage of requiring several weeks to isolate and identify the cultures.

Evans<sup>2</sup> succeeded in isolating *Bact. abortus* from milk by plating on ordinary lactose agar to which 10 per cent of sterile blood serum was added just before plating. After incubating for four days, the colonies which developed were transferred to nutrient broth containing 1 per cent glycerin and to tubes of whole milk containing litmus.

The other method of study, the inoculation of guinea pigs with the milk, while more reliable, is far from satisfactory, owing to the fact that it takes 8 to 10 weeks for the lesions to develop, and it is probable that the organism must be present in large numbers to cause the characteristic lesions with the 5 c. c. of milk used for inoculation.

<sup>1</sup> Nowak, Jules. Le bacille de Bang et sa biologie. In Ann. Inst. Pasteur, t. 22, no. 6, p. 541-556, pl. 5-7. 1908.

<sup>2</sup> Evans, Alice C. Bacillus abortus in market milk. In Jour. Wash. Acad. Sci., v. 5, no. 4, p. 122-125. 1915.

In studying the presence of *Bact. abortus* in milk it was found necessary to develop new technic in order to study a large number of samples. Knowing that this organism is sometimes present in considerable numbers in milk as it comes from the cow's udder, it was thought that this might indicate an infection of the udder and a consequent local production of antibodies. With this in mind, agglutination and complement-fixation tests were made, using milk and milk serum, instead of the usual method of using blood serum. *Bact. abortus* was used as antigen. The object of this paper is to report upon this method.

#### TECHNIC EMPLOYED

**COMPLEMENT-FIXATION TEST.**—The complement-fixation test as used by Surface<sup>1</sup> and others, was employed in this work. Rennet milk serum was used in the following quantities: 0.1, 0.04, 0.02, and 0.005 c. c. Milk was considered positive only when the tube containing 0.04 c. c. of serum was positive. Preliminary tests run upon samples of milk show that the agglutination and complement-fixation tests correspond closely. For this reason only the results of agglutination tests will be given in this paper.

**AGGLUTINATION TEST.**—Antigen was prepared for the agglutination test by growing a culture of *Bact. abortus* upon ordinary agar for 48 hours. The growth was then washed off with a solution containing 0.9 per cent sodium chlorid and 0.5 per cent phenol. The suspension was then filtered through a coarse filter paper and standardized so that the turbidity compared with tube 1.5 of McFarland's nephelometer.<sup>2</sup> Four c. c. of this bacterial suspension are placed in each of the small test tubes used and the following quantities of milk added: 0.1, 0.05, 0.025, 0.01, and 0.005 c. c. In this way approximate dilutions of 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000 were obtained. It was found that turbidity due to the whole milk added did not interfere with the reading of the reaction. When a dilution lower than 1 to 50 was made, rennet milk serum was used.

For the experiment given in Table I, a cow was selected whose milk had given a negative agglutination reaction since first tested, October 10, 1914, using *Bact. abortus* as antigen. Thirty-five c. c. of a 48-hour broth culture of *Bact. abortus* was introduced into the right rear quarter after it had been milked dry. As shown in the table, the agglutinins had appeared in the right rear quarter the following day and soon spread to the other quarters. This spreading was probably brought about by the organism being carried from quarter to quarter upon the hands during milking. After the cow freshened the reaction was seen to gradually die out.

<sup>1</sup> Surface, F. M. The diagnosis of infectious abortion in cattle. Ky. Agr. Exp. Sta. Bul. 166, p. 301-365, 5 figs. 1912.

<sup>2</sup> McFarland, Joseph. The nephelometer. . . In Jour. Amer. Med. Assoc., v. 49, no. 14, p. 1176-1178, 5 figs. 1907.

TABLE I.—Test showing the appearance in milk of agglutinins for *Bacterium abortus* after the introduction into the cow's udder of a pure culture of *Bact. abortus* Bang<sup>a</sup>

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
Oct. 1914.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feb. 1915.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25.....	(b)	(b)	(b)	(b)	(b)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
26.....	P	P	P	P	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
27.....	P	P	P	P	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mar. 1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9.....	(c)	(c)	(c)	(c)	(c)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Apr. 17.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 26.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 4.....	P	P	P	P	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
July 28.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aug. 21.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sept. 13.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

<sup>a</sup> The +, —, and P signs used in all the tables refer to agglutination reaction in the corresponding tube. For instance, +++P— indicates that agglutination took place in the tubes containing 0.1, 0.05, and 0.025 c. c. of milk, partial agglutination took place in the tube containing 0.01 c. c. of milk, and there was no agglutination in the tube containing 0.005 c. c. of milk.

In all cases, unless otherwise stated, the milk was taken a little before what was estimated to be the middle of the milking.

<sup>b</sup> 35 c. c. of a 48-hour broth culture of *Bact. abortus* introduced into right rear quarter.

<sup>c</sup> Cow calved. Bull calf died on Mar. 13, 1915, owing to undigested curd. Reaction of blood of calf; —agglutination; +complement-fixation test.

Table II gives the history of milk from a cow with a record of frequent abortions. As shown in the table, the isolation of *Bact. abortus* from the milk and the results of guinea-pig inoculation prove the presence of this bacterium, as indicated by agglutination reactions.

TABLE II.—History of milk from a cow with a record of frequent abortions<sup>a</sup>

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
Jan. 1914.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10 <sup>b</sup> .....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Apr. 30.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 5.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 11.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
July 12.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12 <sup>c</sup> .....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aug. 10.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oct. 10.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nov. 19 <sup>d</sup> .....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

<sup>a</sup> Known abortions: Dec., 1909; Jan., 1914. Jan., 1911, last living normal calf. Other records of abortions lost.

<sup>b</sup> Isolated a pure culture of *Bact. abortus* direct from milk.

<sup>c</sup> Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed eight weeks later.

<sup>d</sup> Died; impaction of stomach. No lesions or abnormal conditions found in udder.



In Table III the record of milk from another cow is given. Here again we have positive agglutination coupled with abortions and milk shown to contain *Bact. abortus* by guinea-pig inoculation.

TABLE III.—History of milk from a cow with a record of frequent abortions

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1914.																				
July 20.....	+	P	—	—	—	+	+	P	—	—	+	+	—	—	—	+	P	—	—	—
Aug. 4 <sup>a</sup> .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oct. 1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nov. 27 <sup>b</sup> .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dec. 2.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1915.																				
Jan. 15.....	+	+	P	—	—	+	P	—	—	—	+	P	—	—	—	+	—	—	—	—
Mar. 25.....	P	P	—	—	—	P	P	—	—	—	P	P	—	—	—	P	—	—	—	—
June 8.....	P	P	—	—	—	P	P	—	—	—	P	P	—	—	—	P	—	—	—	—
30 <sup>c</sup> .....	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—
Sept. 10 <sup>d</sup> .....	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—
Oct. 10.....	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—
Nov. 4.....	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—

<sup>a</sup> Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions when autopsies were performed 10 weeks later.

<sup>b</sup> Aborted a 7-month fetus.

<sup>c</sup> Right rear quarter, positive guinea-pig inoculation. Right front quarter lost, and left rear and left front quarters negative.

<sup>d</sup> Aborted a 7-month fetus.

In Table IV is given the record of milk from a cow that has never aborted. On June 16, 1915, agglutinins had appeared in all but the left front quarter. Guinea-pig inoculations made on June 30 were positive for infectious abortion in all but the left front quarter. On October 16, 1915, the reaction had spread to the left front quarter. Milk from this quarter is now being tested by guinea-pig inoculation.

TABLE IV.—History of milk from a cow that has never aborted

[Agglutination reaction at middle of milking, when various quantities of milk were added to test tubes containing bacterial suspension]

Date.	Right rear quarter.					Right front quarter.					Left rear quarter.					Left front quarter.				
	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.	0.1 c. c.	0.05 c. c.	0.025 c. c.	0.01 c. c.	0.005 c. c.
1915.																				
Apr. 9.....	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
June 16.....	+	P	—	—	—	+	P	—	—	—	+	—	—	—	—	+	—	—	—	—
30 <sup>a</sup> .....	+	+	P	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—
Oct. 16.....	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—

<sup>a</sup> Guinea pigs inoculated intra-abdominally with milk from each quarter had typical *Bact. abortus* lesions and blood reactions, with exception of left front quarter, which was normal.

While, in Tables I to IV, a positive agglutination test points to the presence of *Bact. abortus* in the milk, this is not always true if judged by guinea-pig inoculation. In several cases the writer was unable to get positive lesions in guinea pigs with milk from all four quarters that gave a positive agglutination reaction. In these instances it is probable that the agglutinins were coming from the blood stream, or, if due to a bacterial invasion of the udder, the bacterium may have been present in too small numbers to cause lesions in guinea pigs with the 5 c. c. of milk used for inoculation. In the instances of agglutination with negative guinea-pig inoculation it was noticed that the reaction from quarter to quarter seemed to be fairly constant. In the tables given, the reaction is seen to vary a good deal from quarter to quarter. This, the writer believes, indicated that in the cases of reaction without pathogenicity to guinea pigs the agglutinins were coming to each quarter from a common source the blood.

Though many samples of milk have been inoculated into guinea pigs, at no time has a sample been found with a negative agglutination test that would produce the typical lesions of infectious abortion.

The present value of this test is that it enables one to select from a herd the cows whose udders may be infected with *Bact. abortus*. The comparatively small number separated by this method may then be examined by guinea-pig inoculation and cultural methods.

If *Bact. abortus* is found to be pathogenic for humans, as has been suggested by Melvin,<sup>1</sup> this test may be of value as another means of safeguarding certified and all unpasteurized milk.

From observations and tests now being made it appears that it may be possible to differentiate samples in which the agglutinins come from the blood from those in which the agglutinins are produced in the udder.

#### SUMMARY.

A pure culture of *Bacterium abortus* Bang introduced into the milk cistern of a cow's udder caused the appearance of agglutinins in the milk.

In every case in which *Bact. abortus* was found present in the milk by animal inoculation the agglutinins for this organism were also found, but this bacterium was not found in every case in which agglutinins were demonstrated.

The agglutination test is of value in studying the presence of *Bact. abortus* in milk when it is desired to study a large number of samples.

If *Bact. abortus* is found to be pathogenic for humans, this test may be of value as another means of safeguarding certified and all unpasteurized milk.

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<sup>1</sup> Melvin, A. D. Infectious abortion of cattle and the occurrence of its bacterium in milk. I.—Introductory statement. In U. S. Dept. Agr. Bur. Anim. Indus. 28th Ann. Rpt. 1912, p. 237-238. 1913.



# BORON: ITS ABSORPTION AND DISTRIBUTION IN PLANTS AND ITS EFFECT ON GROWTH

By F. C. COOK,  
*Physiological Chemist, Bureau of Chemistry*

## INTRODUCTION

The experiments reported in this paper were made in connection with a cooperative study of borax and calcined colemanite as larvicides for the house fly conducted by the Bureaus of Entomology, Chemistry, and Plant Industry, of the Department of Agriculture. The object of this particular study was to determine the effect of boron-treated horse manure on plant growth and to study the absorption of boron and its distribution in the roots, stems, and fruit of plants grown on soil fertilized with this manure and on soil fertilized with untreated manure. The plants were grown in pots in the greenhouses of the Department and on open plots at Arlington Experimental Farm, Va.; Dallas, Tex.; Orlando, Fla.; and New Orleans, La. Analyses of the soil from several treated and untreated plots are included.<sup>1</sup>

Certain deposits of boron have been known for centuries, but the wide distribution of this element in mineral and vegetable matter has been recognized only during the last few years. Probably the first to record the presence of boron in plants were Wittstein and Apoiger (14),<sup>2</sup> who found it in the seeds of *Maessa picta*. Since then many observers have found boron in soils, rocks, fruits, and vegetables.

As soils in many places contain boron, it is not surprising that this element is widely distributed in small amounts in plants. It is also probable that boron is present in nearly all animal material. Bertrand and Agulhon (3) report its presence in the hair, horns, bones, liver, and muscles of animals. They detected boron in 27 species of animals, and state that it probably exists in all animals, being more common in those of marine origin. Boron was also found in human, asses', and cows' milk and in the eggs of the chicken, turkey, and goose.

The toxic effect of boron on plants was first shown in 1876 by Peligot (12), who noted a yellowing of the leaves of beans and reported that in many cases the yellow leaves fell from the plants. The previous year Heckel (8) reported that 1.25 per cent solutions of alkali borate retarded germination for from one to three days, and that 3 per cent of the alkali borate solutions stopped germination entirely. Loew (10, p. 374) states

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<sup>1</sup> The writer desires to express his thanks to Mr. W. D. Hunter, of the Bureau of Entomology, for his material assistance in arranging for the experiments in the South.

<sup>2</sup> Reference is made by number to "Literature cited," pp. 889-890.

that certain algæ, such as *Spirogyra* and *Vaucheria*, are resistant to the action of boron. Morel (11), however, states that very weak solutions of boric acid arrest the development of lower fungi and similar organisms. He suggests that boric acid may be used, like copper, to attack such diseases as mildew and anthracnose. The effect of boron on the lower plants, fungi, yeasts, etc., has been but little studied.

Agulhon (1) and Bertrand (2) have stated that boron in small amounts acts as a stimulant to plant growth. Pellet (12) calls attention to some experiments which indicate that compounds of both manganese and boron, singly and combined, have no effect on the growth or yield of the sugar beet. He concludes that the results of other workers claiming a stimulation are too few and are untrustworthy.

Many investigations regarding the effect of boron on plants and plant growth have been reported, but no attempt to review all such experiments is made in this paper. For a review of this subject the publication of Haselhoff (7) and the recent work of Brenchley (4), where various inorganic plant poisons and stimulants are discussed, should be consulted.

#### EXPERIMENTAL WORK

Very few of the previous studies have included a quantitative estimation of the boron present in plants, and no experiments concerning the effects of calcined colemanite (crude calcium borate) on plant growth have been reported. As both borax and calcined colemanite are valuable larvicides for the house-fly maggot, it seems advisable to determine the effect of manure treated with both borax and calcined colemanite on the growth of a variety of plants.

The manure used in these tests was treated with the amounts of borax or calcined colemanite noted in the tables, and stood in the open for 10 days before it was applied to the soil. For the plot tests, the manure was applied at the rate of 20 tons per acre and was then plowed under, the ground harrowed, and sometimes rolled and reharrowed, before planting. In nearly all of these experiments borax or calcined colemanite was applied to the manure in larger quantities than were required to act as a larvicide—i. e., 0.62 per pound per 8 bushels, or 10 cubic feet. When the manure was mixed with the soil at the rate of 20 tons per acre, 216 pounds of borax per acre were present. Furthermore, the manure was not allowed to stand and leach for longer than 10 days; consequently, practically the entire amount of borax added reached the soil.

When 0.62 pound of borax was applied to each 8 bushels of manure and the weight of 8 bushels of manure estimated at 115 pounds (the average weight of fresh manure containing a large amount of straw), 100 pounds of manure contained 0.54 pound of borax, and when the manure was applied to the soil at the rate of 1 part to 40, the percentage of boron in the soil, calculating the weight of 1 acre of soil 6 inches deep as 1,750,000 pounds, was 0.0015.

Tests with tomato (*Lycopersicon esculentum*) and lettuce (*Lactuca sativa*) were made on plants which had been grown in boxes in green-houses until they were 2 to 3 inches high, when they were transplanted in their respective pots containing the mixtures of manure and soil. The potatoes (*Solanum tuberosum*) tested were of the Green Mountain variety and the seeds used in growing the other plants were common varieties. The percentages of boric acid as recorded in the tables are calculated to a water- and ash-free basis. At least four pots for each treatment were employed in the pot tests. The plots at Arlington Farm were one-twentieth of an acre and those in the South about one-sixtieth of an acre in size. The tests with lettuce were carried out in benches, each 3 by 5 feet.

#### DESCRIPTION OF METHODS

Many tests for determining boron in foods and other material have been devised. When small amounts are present, as was the case in the present experiments, it is determined colorimetrically, using curcumin, the active principle in turmeric (*Curcuma longa* L.), a characteristic red color being given when boron is present.

In preparing the samples, the roots were separated from the plants. Both roots and plants were washed, dried, and cut into small pieces for analysis. In some cases the fruit also was tested. In such instances it was washed, dried, and ground for analysis. Boron was determined by the use of freshly prepared strips of curcumin paper, prepared by immersing large unfolded filter paper in a 0.2 per cent alcoholic solution of curcumin. The procedure was as follows: About 3 gm. of a dried sample were treated with sufficient saturated lime water to make the reaction alkaline. After a thorough mixing in platinum dishes, the samples were dried and heated in a muffle until all of the organic matter had burned off. Ten c. c. of water and a little hydrochloric acid were added and the solution was warmed, filtered, washed, and made to 100 c. c. volume. A 50 c. c. aliquot was usually taken for the determination of the boron, but this varied according to the amount present. To the 50 c. c. aliquot, or a smaller aliquot diluted to 50 c. c., placed in small porcelain evaporating dishes, 2 c. c. of hydrochloric acid were added, and strips of curcumin paper were suspended and allowed to dip into those solutions to the depth of one-fourth of an inch. In all cases standard boric-acid solutions, as well as blanks, were simultaneously employed. After four hours the colors on the strips of paper were compared and the percentage of boric acid determined.

In the case of soils, the boron soluble in weak hydrochloric acid, not the total boron, was determined. Fifty gm. of soil were shaken with 200 c. c. of a solution of hydrochloric acid (1:20) for one hour. This was filtered and 100 c. c. of the filtrate made alkaline with lime water, evaporated to dryness, and ashed. The ash was acidified with hydrochloric

acid and the solution made to 100 c. c., a 50 c. c. aliquot being used for the colorimetric test. In some cases larger amounts of soil were used for the tests. From 2 to 3 gm. of the plant samples were used for moisture and ash determinations.<sup>1</sup>

### RESULTS OF EXPERIMENTS

The results of the experiments are expressed in all the tables and text as percentages of boric acid. Some analyses of boron soluble in weak hydrochloric-acid extracts of soils are also reported. The form of the combination of the boron in plants is not known. The boron of soils is in part present in insoluble combinations with silica, and the absence of acid-soluble boron in some soils may be thus explained. Ash results are also reported for most of the plants analyzed. Separate analyses of the tops, roots, and fruits are tabulated.

In Table I analyses showing the distribution of ash and boron in the tops and roots of wheat (*Triticum* spp.) and beets (*Beta vulgaris*) 3 months old, grown in the presence of calcined colemanite and borax, with and without the addition of lime, are recorded. More boron was found in the tops than in the roots of both plants. The beets absorbed more boron than the wheat plants, especially from the soil treated with calcined colemanite. All of the control plants contained a little boron.

TABLE I.—Percentage of boron in wheat and beets: Greenhouse pot tests<sup>a</sup>

Series No.	Treatment of manure per 8 bushels.	Wheat (dry basis).				Beets (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.	Ash.	Boron as boric acid, ash-free basis.
1	0.75 pounds of calcined colemanite added.....	Per ct. 15.55	Per ct. 0.0103	Per ct. 20.00	Trace.	Per ct. 23.10	Per ct. 0.0315	Per ct. 17.74	Per ct. 0.0020
2	1.5 pounds of calcined colemanite added.....	12.96	.0097	24.76	Trace.	21.69	.0402	12.75	.0025
3	1 pound of borax added.....	12.58	.0097	33.48	0.0008	22.39	.0120	14.58	.0054
4	1 pound of borax and 1 ounce of lime added.....	8.51	.0122	23.39	.0029	23.07	.0172	.....	.0097
5	1 pound of borax and 3 ounces of lime added.....	9.63	.0105	25.69	.0044	22.77	.0154	14.12	.0087
6	1 pound of borax and 9 ounces of lime added.....	11.07	.0173	26.24	Trace.	20.56	.0062	14.41	.0047
7	Control.....	9.20	.0013	23.76	Trace.	23.80	Trace.	14.56	.0013

<sup>a</sup> Forty parts of soil and 1 part of boron-treated manure were mixed in all the pot and bench tests.

A similar series of tests using tomatoes and cowpeas (*Vigna sinensis*) are recorded in Table II. The number and weight of the tomatoes obtained from four pots, which are also recorded, show the injurious

<sup>1</sup> The analyses were completed with the assistance of Mr. J. B. Wilson, of the Animal Physiological Chemical Laboratory, to whom the writer desires to express his indebtedness.

action of the boron alone and the benefit derived from adding lime. The tops of the tomatoes contained rather a large quantity of boron, the roots and fruit but traces. More boron was absorbed by the tomato plants when borax was added than with the addition of calcined colemanite. The addition of lime with the borax retarded the absorption of boron. The lowest percentage of dry matter was found in the tomatoes grown on the soil where borax alone was added. The tops of the control plants contained the least ash.

TABLE II.—*Boron in tomatoes and cowpeas: Greenhouse pot tests*

Series No.	Treatment of manure per 8 bushels.	Tomatoes.							
		Tops.		Roots.		Fruit.		Yield of fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (ash-free basis).	Number.	Weight.
1	0.75 pound of calcined colemanite added.	<i>Per ct.</i> 13.13	<i>Per ct.</i> 0.0054	<i>Per ct.</i> 9.59	<i>Per ct.</i> Trace.	<i>Per ct.</i> 6.04	<i>Per cent.</i> Faint trace.	17	<i>Ounces.</i> 37.25
2	2.5 pounds of calcined colemanite added.	14.44	.0107	10.86	..do...	5.94	....do....	16	31.5
3	1 pound of borax added.....	11.87	.0146	28.80	None.	4.72	....do....	10	10
4	1 pound of borax and 1 ounce of lime added.	12.95	.0123	13.76	..do...	....	....do....	15	33
5	1 pound of borax and 3 ounces of lime added.	12.15	.0072	10.66	..do...	5.26	Faint trace.	17	34
6	1 pound of borax and 9 ounces of lime added.	12.00	Trace.	19.43	..do...	5.85	....do....	18	35
7	Control.....	10.12	..do...	21.88	Trace.	5.92	....do....	23	40.25

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).					
		Tops.		Roots.		Fruit.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
1	0.75 pound of calcined colemanite added.	<i>Per cent.</i> 9.27	<i>Per cent.</i> 0.0339	<i>Per cent.</i> 18.52	<i>Per cent.</i> 0.0033	<i>Per cent.</i> 3.68	<i>Per cent.</i> 0.0135
2	2.5 pounds of calcined colemanite added.	9.25	.0287	27.04	Trace.	3.90	.0106
3	1 pound of borax added.....	8.54	.0243	24.40	None.	3.36	.0133
4	1 pound of borax and 1 ounce of lime added.	10.96	.0115	10.01	....do....	4.12	.0222
5	1 pound of borax and 3 ounces of lime added.	10.08	.0237	17.44	....do....	3.01	.0097
6	1 pound of borax and 9 ounces of lime added.	11.36	.0302	20.64	.....	3.40	.0029
7	Control.....	7.84	.0068	22.58	None.	3.20	.0094

The tops of the cowpeas contained the most boron and the roots the least, the fruit being intermediate. The addition of lime with the borax did not influence the total amount of boron absorbed by the plants. The control cowpeas contained larger amounts of boron than the tomato control plants. The tops of the control cowpeas contained the least ash.



The results of the greenhouse, bench, and pot tests with lettuce and tomatoes are recorded in Table III. It is evident that the lettuce plants took up boron in proportion to the amounts present in the soil. The control lettuce contained the lowest percentage of solids and indicated the presence of boron. A slight chlorosis of the lettuce plants grown in series 1 and 2 was seen, but no injury to the roots was observed. The results of the analyses of the upper and lower 6 inches of soil in the benches show an even distribution of the boron.

TABLE III.—*Boron in lettuce and tomatoes: Greenhouse bench and pot tests*

Series No.	Treatment of manure per 8 bushels.	Lettuce (entire plant).		Soluble boron as boric acid in soil on which lettuce was grown.	
		Dry matter.	Boron as boric acid (dry basis).	Upper 6 inches of soil.	Lower 6 inches of soil.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	0.75 pound of borax added.....	11.6	0.00036	0.0012	0.0010
2	1.25 pounds of borax added.....	10.0	0.00064	0.0022	0.0008
3	Control.....	9.0	0.00020	Faint trace.	Faint trace.
4	0.5 pound of borax added.....		0.00036		
5	0.62 pound of borax added.....		0.00042		
6	0.75 pound of borax added.....				
7	Control.....		0.00015		

Series No.	Treatment of manure per 8 bushels.	Tomatoes.					
		Tops (dry basis).		Fruit (fresh basis).		Yield.	
		Ash.	Boron as boric acid (ash-free basis).	Dry matter.	Boron as boric acid (water and ash free basis).	Number.	Weight.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>		<i>Ounces.</i>
1	0.75 pound of borax added.....	12.98	0.0089	6.55	Faint trace.	123	157
2	1.25 pounds of borax added.....	12.94	0.0196	6.60	do.....	101	130 $\frac{1}{2}$
3	Control.....	10.10	0.0009	6.75	.....do.....	120	153 $\frac{1}{2}$
4	0.5 pound of borax added.....	10.01	0.015	8.10	0.0002		
5	0.62 pound of borax added.....	10.77	0.016	8.01	0.0004		
6	0.75 pound of borax added.....	7.72		7.51	0.0003		
7	Control.....	7.72	0.0013	8.00			

Tomato plants 1, 2, and 3, Table III, were 6 months old at the time of analysis. The yield of fruit from three pots in each series, 1, 2, and 3, showed no reduction in the case of the 0.75-pound borax application, but the 1.25-pound borax application reduced the yield. The dry matter of the control fruit, series 3, is higher than in series 1 or 2, and the ash of the control tops, series 3, is lower than the ash for the tops, series 1 and 2. The tomato plants, series 4, 5, 6, and 7, were younger and smaller than those of series 1, 2, and 3. In all the tomato plants (Table III) the tops contained practically all the boron, the fruit showing only traces.

The results with wheat grown in plots at Arlington Farm, Va., are given in Table IV. The manure was applied at the rate of 20 tons per acre. The wheat was planted in October, 1913, and harvested in June, 1914, the soil samples being tested at the time of harvesting. On the borax plot the wheat plants which were yellow during the winter, became green and normal in appearance in the spring. The yield of wheat from the borax plot was 90 per cent of the control, but larger than that from an unmanured plot which was simultaneously tested. The amount of borax added to the borax plot was about four times that necessary to act as a larvicide, but only a trace of boron was found in the wheat grain or straw. The wheat grains were sound and the nitrogen and ether-extract results of the control differed very little from those of the wheat and straw from the borax-treated plot. A trace of boron was found in the grains and straw from the borax plot, and the borax-treated soil showed 0.003 per cent of boric acid. The soil sample from the borax plot contained more nitrates than the control sample. Nitrogen was estimated by the Kjeldahl-Gunning method, and nitrates by the method recommended by the American Public Health Association.

TABLE IV.—Percentage of boron in wheat, straw, and soil: Plot tests at Arlington Farm, Va.

Series No.	Treatment of manure per 8 bushels.	Material.	Nitrogen.	Nitrogen as ammonia (MgO method).	Nitrogen as nitrates.	Ether extract.	Acid-soluble boron as boric acid.
1	2 to 3 pounds of borax added.	Wheat grains.....	2.15	.....	.....	1.70	Faint trace.
		Wheat straw.....	.381	.....	.....	2.12	Do.
		Soil 3 to 4 inches deep..	.09	0.004	0.0018	.....	0.003.
2	Control.....	Wheat grains.....	2.21	.....	.....	1.77	None.
		Wheat straw.....	.323	.....	.....	2.27	Do.
		Soil 3 to 4 inches deep..	.09	.003	.0012	.....	Do.

Results of the analyses of soybeans (*Glycine hispida*), string beans (*Phaseolus vulgaris*), and potato plants grown on plots at Arlington Farm, Va., are recorded in Table V. The roots and beans of the soybeans contained about equal amounts of boron, and rather large quantities were found in the tops of all the plants analyzed. There was a more equal distribution of boron in the roots, tops, and beans of the string beans than in the case of the soybeans.

The potatoes showed only traces of boron in the tops, the largest part of the boron being found in the roots, although the tubers contained a fairly large amount. All control plants contained a little boron. The addition of lime with the borax did not prevent the absorption of boron by the plants, as much boron being absorbed from the calcined-colemanite plots as from the borax plots.

TABLE V.—Percentage of boron in soybeans, string beans, and potatoes: Plot tests at Arlington Farm, Va.

Series No.	Treatment of soil per square rod.	Boron as boric acid (dry basis).								
		Soybeans.			String beans.			Potatoes.		
		Roots.	Tops.	Beans.	Roots.	Tops.	Beans.	Roots.	Tops.	Potatoes.
1	1.62 pounds of calcined colemanite added . . . . .	0.0086	0.0048	0.0092	0.0044	0.0075	0.0045	0.0170	0.0012	0.0094
2	2.88 pounds of calcined colemanite added . . . . .	0.0160	0.0076	0.0136	0.0083	0.0177	0.0213	0.0144	Trace.	0.0022
3	3.96 pounds of borax added . . . . .	0.0124	0.0047	0.0104	0.0086	0.0093	0.0117	0.0354	do . . .	0.0066
4	3.96 pounds of borax and 2 pounds of lime added . . . . .	0.0126	0.0040	0.0164	0.0093	0.0099	0.0080	0.0165	do . . .	0.0019
5	2 pounds of lime added . . . . .	0.0030	0.0008	0.0036	0.0050	None.	0.0042	Trace.	None.	0.0010

In Table VI results of the analyses of corn (*Zea mays*), wheat, peas (*Pisum sativum*), and oats (*Avena sativa*), grown on plots at New Orleans, La., and Dallas, Tex., are recorded. The entire plants, which were 3 months old and small, were used. The corn and wheat plants took up equal amounts of boron. Soluble boron was found in all nine samples of soil from New Orleans, while only two of the five samples from Dallas contained any. The peas absorbed more boron than the oats, especially in series 1, 2, and 3.

TABLE VI.—Percentage of boron in corn, wheat, peas, oats, and soil: Plot tests at New Orleans, La., and Dallas, Tex.

Series No.	Treatment of manure per 8 bushels.	New Orleans, La.		Dallas, Tex.	
		Boron as boric acid (entire plant, dry basis).		Boron as boric acid (entire plant, dry basis).	
		Corn.	Wheat.	Peas.	Oats.
1	0.5 pound of borax added . . . . .	0.013	0.011	0.0006	0.010
2	0.62 pound of borax added . . . . .	0.015	0.015	0.0009	0.010
3	Control . . . . .	Trace.	Trace.	Trace.	0.006
4	0.75 pound of borax added . . . . .	Trace.	Trace.	0.0003	0.040
5	1.25 pounds of borax added . . . . .	Trace.	Trace.	0.0013	0.026
6	Control . . . . .	Trace.	Trace.	0.0005	0.025
7	0.75 pound of calcined colemanite added . . . . .	Trace.	Trace.	Trace.	Trace.
8	1.50 pounds of calcined colemanite added . . . . .	Trace.	Trace.	Trace.	Trace.
9	Control . . . . .	Trace.	Trace.	Trace.	Trace.

TABLE VII.—Percentage of boron and ash in radishes, string beans, cowpeas, peas, and soil: Plot tests at Orlando, Fla.

Series No.	Treatment of manure per 8 bushels.	Radishes (dry basis).				String beans (dry basis).			
		Tops.		Roots.		Tops.		Roots.	
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).
1	0.75 pound of borax added.....	34.44	0.162	50.08	0.039	.....	0.086	22.98	0.011
2	1.25 pounds of borax added.....	49.49	.226	51.12	.087	17.56	.080	14.89	.015
3	Control.....	45.25	.018	45.04	.010	22.80	.011	.....	.007

Series No.	Treatment of manure per 8 bushels.	Cowpeas (dry basis).				Peas (entire plant, dry basis).	Soluble boron as boric acid found in sample of soil 3 to 4 inches deep.
		Tops.		Roots.			
		Ash.	Boron as boric acid (ash-free basis).	Ash.	Boron as boric acid (ash-free basis).	Boron as boric acid.	
1	0.75 pound of borax added .....	29.49	0.162	35.15	0.222	0.212	0.0006
2	1.25 pounds of borax added .....	33.22	.140	45.68	.240	.229	.0010
3	Control.....	20.18	.024	.....	.029	.024	.0003

In Table VII the boron content of radish (*Raphanus sativus*), string-bean, cowpea, and pea plants, grown on borax and control plots at Orlando, Fla., is given. An appreciable amount of soluble boron was found in the soil samples from all three plots. The radish plants contained a large amount of boron in the tops, as well as an appreciable quantity in the roots. The string beans did not absorb as much boron as the radishes; but contained a large percentage of the absorbed boron in the tops. The cowpeas absorbed large amounts of boron, more being found in the roots than in the tops. The pea plants also absorbed boron in great quantities. All the control plants contained boron to a marked degree, which is not surprising, as 0.0003 per cent of soluble boron was found in the control soil sample examined at the close of the test.

As there was little rain at Orlando while these tests were being conducted, and as relatively large quantities of soluble boron were found in the samples of soil tested, it is not surprising that the plants absorbed large amounts of boron.

#### DISCUSSION OF EXPERIMENTAL WORK

It apparently made little difference in the quantities of boron absorbed by the various plants whether it was added to the manure used on the soil in the form of calcined colemanite or as borax. The addition of

lime to the borax also showed no definite action in preventing the absorption of boron, although with beets (Table I) and with one series of tomatoes (Table II) such a reduction is indicated where the largest application of lime was made. Most of the plants analyzed took up boron in proportion to the amounts present in soluble form in the soil.

The leguminous plants, which were most easily injured by boron, absorbed larger amounts than the other plants tested, while wheat and oats absorbed but little boron. It is particularly noteworthy that the wheat grown at Arlington Farm, Va., on soil fertilized with manure heavily treated with boron showed only traces of boron in the grain and straw. Haselhoff (7) found boron in the stalk of maize, but not in the grain.

The most striking differences in the absorption and distribution of boron are shown by the leguminous plants, where a more even distribution between roots, tops, and fruit is found. Potatoes also showed rather a large quantity of boron in the roots and tubers, but only a small amount in the tops. Succulent plants like beets also absorbed boron. On the other hand, tomatoes and wheat showed only traces of boron in the fruit and but little in the roots. Agulhon (1) has investigated the action of boric acid on wheat, using synthetic sterile liquid media, including both soil and water cultures. He recommends 0.0012 per cent of boron to obtain the best growth. In these tests, when borax was added at the rate of 0.62 pound to each 8 bushels of manure and this manure applied to the soil at the rate of 15 tons per acre, 0.0015 per cent of boron was added to the soil.

The fact that all control plants contained a little boron shows the wide distribution of boron in the soil. From the large amounts taken up by the control plants grown at Orlando, Fla., it appears that the soil there contains more than the soil at Dallas, Tex., New Orleans, La., or Arlington Farm, Va.

The ash results of the various portions of the plants analyzed vary considerably, and the variations are not in a definite direction.

A spotting or yellowing of the leaves of plants, which was first noted by Hotter (9) and later reported by several investigators, was observed in these experiments when boron was present in the soil to any extent. In the case of the tomato plants, Table II, a yellowing of the leaves was noted when borax was used at the 0.75-pound rate, but the yield was unaffected. In some of the legumes—namely, string beans, soybeans, and peas—a noticeable yellowing of the leaves was observed when borax was added at the rate of 0.75 pound, and in these cases a reduction in stand took place. The wheat plants grown at Arlington Farm on the plot fertilized with manure treated with from 2 to 3 pounds of borax to each 8 bushels, as noted on page 883, were yellow during the first 3 or 4 months of growth. When the growth started in the spring, however, the plants became green, and the yield of the grain was 90 per cent of the

control yield, more than that obtained from the unmanured control plot. The yellowing of the leaves is an unmistakable sign of injury, although in some cases the plant can recover, or at least is not sufficiently injured to cause a reduction in the yield.

Haselhoff (7) states that the action of boron is more marked on beans than on oats or corn, and that it can be seen when small amounts of boron are present in the soil and when no action injurious to plant growth is evident. He says further that small amounts of boron stimulate the growth of beans and corn, while large amounts produce injury. In his experiments beans absorbed boron in proportion to the amount present in the soil up to a certain limit. The plants examined by Haselhoff contained from 0.04 to 0.17 per cent of boron, which is more than was found in these experiments, with the exception of the plants grown at Orlando, Fla. (Table VII). He suggests that for safety the amount of boron in the soil be less than 0.0001 per cent. According to Brenchley (5), peas are stimulated by relatively high concentrations of boric acid, but with larger applications of boric acid the toxic action was well marked on the leaves, which tend to become brown and to die in a characteristic manner.

There is some evidence in the literature to indicate that small amounts of boron stimulate plant growth. Brenchley (5) states that below a certain dilution boron tends to produce stronger roots and shoots. Large amounts of boron are known to be toxic to practically all plants, with the exception of certain fungi.

In these experiments, where in most cases more boron was added than was necessary to act as a larvicide, no stimulating action was noted. On the contrary, an injurious action was seen with leguminous plants, which became yellow and did not show a good stand. Tomatoes, beets, lettuce, potatoes, radishes, corn, oats, and wheat appeared normal when grown in the presence of amounts of boron which produced injury to leguminous plants. When borax is added to manure at the rate of 0.62 pound to each 8 bushels and the manure is applied to the soil at the rate of 15 tons per acre, 0.0011 per cent of boron is added to the soil. This quantity of boron may injure leguminous plants, but did not injure the other plants tested, although no stimulation was noted. If the borax-treated manure is mixed with untreated manure, as would be done in many cases, since it is necessary to treat manure with borax to destroy fly larvæ during only a portion of the year, it is possible that the percentage of boron would be sufficiently reduced to bring about a stimulating action on plant growth.

In connection with the stimulating action of boron, it may be mentioned that nitrites and nitrates were detected in three or four borax-treated manure piles at New Orleans (6, p. 19), while the corresponding control piles contained no nitrites or nitrates, and several soils fertilized with borax manure have shown more nitrates than the check soils. A

stimulating action of boron on the nitrifying bacteria seems to follow in certain cases.

The results at Orlando, where the same amounts of boron were added to the soil as at other points, but where the toxic action of the boron was marked and where soluble boron was found in the soils after several months, indicates that many factors are involved in the absorption of boron and its effect on plants, and that definite conclusions in studies of this nature should be drawn with great care. These results are submitted as a preliminary study of this question. It is our purpose to test the cumulative action of boron in soils.

#### SUMMARY

(1) It apparently made little difference in the quantity of boron absorbed by the plants tested whether boron was added to the soil as borax or as calcined colemanite. The addition of lime with borax had no definite effect in preventing the absorption of boron. Wheat and oats absorbed very little boron, while leguminous and succulent plants absorbed comparatively large amounts.

(2) Wheat, beets, cowpeas, and tomatoes grown in pots in the greenhouses contained boron principally in the tops of the plants, and, with the exception of the beets, comparatively little or none in the roots.

(3) The fruit of the tomato plants contained only traces of boron, while the fruit of the cowpea contained large quantities. Lettuce grown in the greenhouse absorbed boron in proportion to the amounts present in the soil.

(4) Potatoes grown in the open showed, when mature, a small amount of boron in the tops and relatively large amounts in the roots and tubers.

(5) The leguminous plants, string beans, soybeans, and cowpeas, which were very sensitive to boron, showed when grown in plot tests a more equal distribution of the boron among the roots, tops, and fruit than the other plants tested.

(6) Radishes grown in plots contained much larger quantities of boron in the tops than in the roots. Analyses of entire plants of wheat, corn, peas, and oats grown on plots in the South showed the absorption of boron in all cases, the peas absorbing the most. All of the control plants contained at least a trace of boron.

(7) Samples of soil from some of the control plots showed the presence of acid-soluble boron, while several similar samples of soil from certain boron-treated plots showed no acid-soluble boron. Usually more soluble boron was found in the treated soil than in the control soil.

(8) The yield of wheat from a plot heavily treated with borax was 90 per cent of the manured-control yield and greater than the yield from the unmanured control. The wheat grains were sound and contained but a trace of boron.

(9) The yield of tomatoes in pot tests was unaffected when borax was added in amounts to produce 0.0018 per cent of boron in the soil, but when the amount was increased to 0.0030 per cent, a reduced yield resulted.

(10) Numerous factors influence the absorption, distribution, and action of boron in plants.

(11) No more than 0.62 pound of borax or 0.75 pound of calcined colemanite should be added to each 10 cubic feet of manure, and when using the boron-treated manure in growing leguminous plants, the manure should be mixed with untreated manure before being applied to the soil. For other plants, boron-treated manure should not be used at a higher rate than 15 tons per acre.

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# FURTHER STUDIES ON PEANUT LEAFSPOT

By FREDERICK A. WOLF,

*Plant Pathologist, Alabama Agricultural Experiment Station*

## INTRODUCTION<sup>1</sup>

A report of investigations of certain fungous diseases of peanuts has previously<sup>2</sup> been made. Since the appearance of that report the investigations have been continued for the purpose of obtaining additional data on certain phases of the work. Opportunity had not been afforded prior to the present year to test under field conditions the efficacy of rotation and seed treatment in the control of leafspot, *Cercospora personata* (B. and C.) Ellis. Definite experimental data upon the agencies concerned in the distribution of leafspot had not been secured; neither had an effort been made to definitely correlate the destructiveness of the disease with the presence of certain climatic conditions. It was the primary purpose of the present work to secure information upon these phases of the subject. The results of these studies are, therefore, recorded as additions to the information contained in the previous publication<sup>3</sup> upon investigations which were begun four year ago under the Adams fund.

## ROTATION TESTS FOR LEAFSPOT CONTROL

Because of the fact that the leafspot organism was found to live on fallen leaves in the field from one season to the next,<sup>4</sup> it was recommended as a rational method of control that the same fields be not planted to peanuts in successive years. Observations on the effectiveness of rotation were made at several widely separated points in the State, with the representative results which are recorded in Table I.

In many cases it has been difficult to get reliable information as to the crops previously grown upon the fields in which these studies were made, since the tenants knew nothing of the system of cropping employed prior to their tenure. In determining the percentage of plants affected, the

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<sup>1</sup> The writer received valuable aid in the field tests from R. C. Lett, farm adviser for Tuscaloosa County, Ala., on whose farm the seed-treatment tests were conducted, and from S. A. Wingard, who carefully and arduously assisted in the field studies. Indebtedness is hereby acknowledged to both gentlemen for these several services.

<sup>2</sup> Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 127-150, 5 pl. 1914. Bibliography, p. 148-149.

<sup>3</sup> Wolf, F. A. Op. cit.

<sup>4</sup> When these leaves [diseased leaves which had remained out of doors from November until May] were kept moist as when placed in moist chambers, conidia were abjoined. Additional evidence that the fungus remains viable is to be found in the fact that leaf spots developed during May, on young plants, in a field which had grown a badly diseased crop the previous season. (Wolf, F. A. Op. cit., p. 135.)

total number of plants on a certain area was first counted, and then a count was made of those plants which were diseased. A plant having only a single spot on one of its leaves was regarded as diseased. Several attempts were made to determine the decrease in yield due to leafspot, but no satisfactory method has been found and the figures given are only approximate, since they were obtained by determining the average difference in the number of peas borne on 10 healthy and 10 diseased bunches having apparently the same-sized tops. It will be noted that the percentage of diseased plants in fields designated as 1 to 7, which are representative of rotations, varies from 13.5 to 100 per cent. When the results for the fields numbered 4 and 8 are contrasted, the former having borne no peanuts previously for 11 years and the latter having grown four successive crops, with 95 and 100 per cent, respectively, of the plants diseased, with practically no difference in the severity of attack, one is forced to conclude that rotation in itself is not to be regarded as a control measure against peanut leafspot. These results came somewhat as a surprise to the writer. Several reasons for the inefficacy of rotation as a means of leafspot control will be brought out later in this report. It might be suggested at this point, however, that this much overworked and overrecommended suggestion for the control of plant maladies is not a panacea, but requires experimental proof for each particular trouble for which it is recommended.

TABLE I.—Summary of rotation tests with peanuts made in Alabama in 1915

Field No.	Location.	Previous crops on soil.	Date of examination.	Plants affected.	Decrease in yield of peas.
1	Auburn...	Peanuts had been grown 2 years before.....	Sept. 6	<i>Per cent.</i> 100	<i>Per cent.</i> (a)
2	Eutaw...	Peanuts had not been grown for several years previously.	Aug. 28	54	5
3	...do.....	Peanuts had not been grown for 4 or 5 years previously.	30	41	4-5
4	.. do.....	No peanuts had been planted for at least 11 years...	Sept. 31	95	19-5
5	...do.....	No peanuts had been planted the previous year; no previous record available.	1	26	(a)
6	.. do.....	No peanuts in field the previous year.....	1	100	(a)
7	...do.....	No peanuts in field for 4 years previously.....	1	13.5	(b)
8	...do.....	Peanuts had been grown during each of the 4 preceding years.	Aug. 27	100	20

a Not estimated.

b Negligible.

## SEED DISINFECTION FOR LEAFSPOT CONTROL

Seed treatment for the control of leafspot was recommended in a previous report<sup>1</sup> for two reasons. It had been found that conidia adhere to the surface of the shells, and it had been noted repeatedly that the disease occurs in fields not previously planted to peanuts. It was suggested that solutions of copper sulphate or formaldehyde be used in dis-

<sup>1</sup> Wolf, F. A. Op. cit., p. 134. "The prevalence of leaf spot in lands not previously cultivated is not uncommon . . . conidia and conidiophores have been found in the centrifuged washings of peas."

infecting. In case the former was employed, the peas were to be immersed for 15 minutes in a solution containing 1 pound of copper sulphate to 20 gallons of water; in case the latter was used, 1 pint of formaldehyde to 20 gallons of water, the peas to be steeped for an hour. Tests of the effectiveness of these seed treatments were made during the past season (1915) at Eutaw, Ala. One field, designated as field 10, had previously grown several successive crops of peanuts; the other, field 11, had not been cropped with peanuts at least during the four preceding years. Each field was divided into four plots. Plot 1 in each field was planted with unshelled peanuts which had been immersed in copper sulphate; those in plot 2 were not shelled and were immersed in formaldehyde; those in plot 3 were given no treatment; in plot 4 no fungicide was employed, and the peanuts were shelled prior to planting. The conditions in field 10, as noted in three successive examinations, are given in Table II.

TABLE II.—Summary of results of leafspot tests on field 10 in 1915, infested with leafspot

Plot and treatment.	Aug. 6.	Aug. 14.	Aug. 21.
<b>Plot 1. Peanuts, not shelled, steeped in copper sulphate:</b>			
Total number of diseased leaves on 25 plants.....	882	1,492	3,201
Number of diseased leaves per plant—			
Maximum.....	79	110	273
Minimum.....	10	11	23
<b>Plot 2. Peanuts, not shelled, steeped in formaldehyde:</b>			
Total number of diseased leaves on 25 plants.....	755	1,437	3,020
Number of diseased leaves per plant—			
Maximum.....	63	144	241
Minimum.....	3	5	29
<b>Plot 3. Peanuts, not shelled, no treatment:</b>			
Total number of diseased leaves on 25 plants.....	1,022	2,028	4,234
Number of diseased leaves per plant—			
Maximum.....	94	178	297
Minimum.....	7	12	39
<b>Plot 4. Peanuts, shelled, no treatment:</b>			
Total number of diseased leaves on 25 plants.....	545	1,184	2,749
Number of diseased leaves per plant—			
Maximum.....	45	128	204
Minimum.....	2	15	26

Infections were observed in this field as early as July 26 and were probably present at a much earlier date. Plot 3 will be seen to have had a larger number of diseased leaves on August 6, and during the two successive weeks, than did any of the other plots. It would naturally follow from this that seed disinfection is not without appreciable effect. It was felt, however, that it would be necessary to duplicate these results in several localities during several seasons before one could safely conclude that seed treatment is of any practical value, especially in the light of the data to be subsequently presented.

Field 11, which can be directly contrasted with field 10, really shows the result of seed treatment coupled with rotation. No tabulation for field 11, such as has been made for field 10, has been prepared, but the important facts in regard to this field are as follows: Leafspot was not apparent until August 6, 11 days after it was first seen in field 10. Only five plants in the whole field were found to be affected on this date, and

the disease was evidenced by only one or two spots on each leaf. Two of these plants were found in plot 1, one in plot 2, and two in plot 4. It will be noted that on this date plot 1 of field 10 had a maximum of 79 affected leaves on a single plant, plot 2 had 30, plot 3 had 94, and plot 4 had 45. A final count of the number of diseased leaves in field 11 was made on September 1, with the result that 12 per cent of the leaves in plot 1 were affected, 11 per cent in plot 2, 15 per cent in plot 3, and 14 per cent in plot 4. It should be said in explanation that none of these plants had more than six affected leaves, and most of them had only one or two, upon which there were at most only a few spots. Ten days prior to this a final count on field 10 showed a minimum of 23 diseased leaves per plant and a maximum of 297. This number would, no doubt, have been considerably greater by September 1.

The most significant conclusion that one is forced to make from these tests is that seed treatment, either by itself or in conjunction with rotation, does not eliminate peanut leafspot. This conclusion is further supported by the results obtained from the rotation tests given in Table I. The peas used in planting fields 1, 2, 5, 6, and 7 were shelled prior to planting, thus eliminating the danger of introducing infective material at the time of planting. In these fields, 100, 54, 26, 100, and 13.5 per cent, respectively, of the plants were affected with leafspot. The peas used in planting fields 3 and 4 were not shelled, and 41 and 95 per cent, respectively, of the plants were diseased. As can readily be seen from these figures, the removal of the shells prior to planting contributed nothing toward keeping the crop free from disease.

#### DAMAGE SUSTAINED BY PEANUT PLANTS AS A RESULT OF LEAFSPOT

In order to measure the degree to which leafspot affects the foliage of peanuts, an effort was made to determine the relation between the total leaf area and the diseased area of a peanut plant. The plant used was taken from field 10 and may be regarded as a plant having an average proportion of diseased tissues. The method employed consisted in weighing pieces of paper corresponding in area to the total and the diseased leaf surface. From paper of good quality, pieces, each equal in area to one of the leaves of the plant, were cut. After these had been weighed, areas corresponding to the diseased parts of the leaves were outlined, and these areas were then removed. The paper leaf areas with the excised diseased areas were again weighed with the following computed results: The total weight of the leaves on a single plant is found to be 64.07 gm. Of this weight, 20.10 gm. are wholly free from spots; 12.39 gm. are dead as a result of the attacks of *Cercospora personata* and have for the most part fallen off; the remainder, 31.58 gm., are regarded as diseased leaves. Of these diseased leaves 10.18 gm., or 32.04 per cent, are occupied by the fungus. When 12.39 gm. and 10.12 gm. are combined, it is found that 35.07 per cent of the entire leaf area is lost to photosynthetic activity. It

is realized, of course, that these figures represent only an approximation, because the method itself is inexact. It is believed, however, that the approximated losses in yields of from 5 to 20 per cent given in Table I are reasonable, when one considers that there has been a loss to the plant of about 35 per cent in its active leaf area.

#### TESTS ON DISSEMINATION OF LEAFSPOT BY AIR AND WIND

Previous work on air currents as an agency in the dispersal of the leaf-spot fungus yielded only negative results.<sup>1</sup> It was believed, however, in spite of this negative evidence, that conidia are carried short distances by the wind.

The purpose of the tests herein reported was not only to determine whether or not the wind acts as an agent in dissemination of the conidia of *Cercospora personata*, but also to ascertain the conditions of temperature and humidity which might influence its maximum or minimum prevalence in the air. The tests were conducted at Eutaw and Auburn, Ala. The tests at Eutaw, Ala., at which place 210 exposures of plates were made, covered the entire period, nights as well as days, from August 9 to August 26, with the exception of August 15 and August 22. The tests at Auburn, Ala., were conducted from September 6 to September 11 and were made to substantiate the tests made at Eutaw, Ala.

The method formerly employed consisted in the exposure for varying lengths of time of sterile agar in Petri dishes. This method is open to objection for the reasons that at certain times the conidia of *Cercospora personata* germinate poorly or not at all and the development of colonies proceeds so slowly that they are likely to be obscured by more rapidly developing forms. It was decided, therefore, to use essentially the method employed by Burrill and Barrett<sup>2</sup> in their study of the dispersal of *Diplodia zaeae*. Stations 2, 4, 6, and 8 feet distant from the nearest peanut plant were established. A frame to hold the exposure plates in a vertical position about 8 inches from the ground was made. This frame could be moved at the beginning of each exposure, to permit the plates to face toward the prevailing wind. Glass plates 4 by 5 inches were smeared with glycerin only on the side directed toward the peanut plants. Four sets of exposures of three hours duration each were made during the period from 6 a. m. to 6 p. m. One set of exposures of 12 hours duration was made nightly from 6 p. m. to 6 a. m. Rains interfered somewhat with this routine. Plates exposed during a rain were washed off, and those exposed in the periods following rain were found to be free from conidia. Readings of the temperature and relative humidity were made at the beginning of each set of exposures. The

<sup>1</sup> "All attempts to gain definite data showing that the wind is a carrier of the conidia have thus far been unsuccessful." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 134. 1914.)

<sup>2</sup> Burrill, T. J., and Barrett, J. T. Ear rots of corn. Ill. Agr. Exp. Sta. Bul. 133, p. 63-109, 11 pl. 1909.

exposed plates were brought into the laboratory as soon as possible after collection, were placed edgewise in a glass funnel, and the glycerin and contents washed off into a vial with a 2 c. c. pipetteful of 95 per cent alcohol. The stream of alcohol used in washing the plates was permitted to play slowly along the upper edge. The washings were then permitted to evaporate until only a few drops remained in the vials. By examination with the low-power lens of a microscope the number of conidia in these few drops could then be determined.

This method is open to two serious objections. Many of the spores were not washed from the plate by this method, as evidenced by a test in which a plate washed according to the method described and found to have entrapped three conidia of *Cercospora personata* was afterwards washed, using a wash bottle as a means of driving a stream of 95 per cent alcohol forcibly against it, and was found to have nine additional conidia. The other objection, which was encountered by Heald, Gardner, and Studhalter,<sup>1</sup> consists in the fact that it is practically impossible to spread a film of glycerin uniformly on a glass slide and have it remain so for three hours. The results shown in Table III are therefore not representative of the number of conidia that were actually entrapped, but convincingly prove that the conidia of *C. personata* are wind borne.

TABLE III.—Results of tests of glycerin plates exposed to air currents at Eutaw, Ala.

Date of exposure.	Number of plates exposed.		Number of plates with adhering conidia.		Rainfall.	Maximum number of conidia of <i>Cercospora personata</i> on any plate.	Total number of conidia entrapped during the entire period.	
	Day.	Night.	Day.	Night.			Day.	Night.
Aug. 9.....	12	3	5	1	Inches. 0	4	15	2
10.....	12	3	4	0	1.58	3	10	0
11.....	12	3	5	0	0	4	11	0
12.....	12	3	5	1	0.43	4	15	2
13.....	12	3	10	0	0	4	24	0
14.....	12	3	8	1	0.18	4	16	2
15.....	12	3	2	0	2.00	2	3	0
16.....	12	3	2	0	0.37	1	2	0
17.....	12	3	6	1	0	3	12	1
18.....	12	3	5	0	0.04	4	9	0
19.....	8	2	2	0	0.13	3	4	0
20.....	8	2	4	0	0	2	7	0
21.....	8	2	3	1	0.02	3	5	1
22.....	8	2	3	1	1.12	3	5	1
23.....	8	2	3	1	0	3	5	1
24.....	8	2	5	0	0	2	5	0
Total.....	168	42	72	6	.....	.....	150	9
Total day and night.....	210		78		.....	.....	159	

It is not deemed necessary to give a detailed daily record of the actual routine pursued. It will be seen that only 78 of the 210 plates exposed

<sup>1</sup> Heald, F. D., Gardner, M. W., and Studhalter, R. A. Air and wind dissemination of ascospores of the chestnut-blight fungus. In Jour. Agr. Research, v. 3, no. 6, p. 493-526, pl. 63-65. 1915. Literature cited, p. 526-526.

were found to have adhering conidia. The usual number found was three or four on each plate. The occurrence of rain and heavy dews will in part account for the relatively small number of plates upon which conidia were found. Rain fell on 9 of the 16 days during which these tests were made. The plates washed off by these rains numbered 26. Three sets of exposures of three plates each remained free from conidia in the periods immediately following rain. In many cases one plate only of each set gave positive evidence in the period following. Only six out of the 42 plates exposed at night yielded any positive results, owing principally to the occurrence of dews.

At no time during the period in which these tests were made, as will be seen, was there a maximum period of spore dispersal. Conidia were present in the air, except where it had been rendered free from them by precipitation, during the entire period. This is in accord with the increase in amount of leafspot shown in the successive counts made in field 10 and recorded in Table II. There was approximately twice as much leafspot in field 10 on August 14 as on August 6, and twice as much on August 21 as on August 14. No correlation between these increases and the temperature and humidity records could be discovered, and these figures have consequently been omitted from Table III. The idea formerly entertained<sup>1</sup> that the occurrence of peanut leafspot is correlated with certain moisture and temperature conditions is now regarded as without foundation. Such a correlation would be meaningless in view of the positive evidence, next to be reported, that insects act as carriers of leafspot. Details of the tests conducted at Auburn, Ala., are not tabulated, since the work accords with the work done at Eutaw, Ala., and substantiates the significant fact that air currents are agents in the dissemination of *Cercospora personata*.

#### INSECTS AS AGENTS IN DISSEMINATION OF THE LEAFSPOT ORGANISM

The fact that the leafspot fungus is air-borne explains in part at least the failure to secure perfect control in the tests in which rotation and seed treatment were combined. No tests have been made, however, upon the distance which the conidia may be transported by the wind. The most distant exposures were only 8 feet from the nearest diseased plant. It seems unlikely that air dispersal could account for severe infection in fields in which both rotation and seed treatment had been practiced and which were from  $\frac{1}{4}$  to  $\frac{1}{2}$  mile distant from the nearest infected field. It was therefore suspected that certain insects, among which grasshoppers are the most important, are agents in this spread of leafspot.

<sup>1</sup> "Apparently infection with *Cercospora* is in some manner correlated with certain moisture and temperature conditions. . . The ravages of *Cercospora personata* seem to attain their maximum severity after a dry period followed by excessively sultry weather. . ." (Wolf, F. A. Leafspot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 133. 1914.)



A relatively meager literature dealing with the subject of insects as carriers of fungi producing plant diseases has accumulated. Since the most important publications upon this subject are summarized in a recent excellent paper by Studhalter and Ruggles,<sup>1</sup> an historical review is purposely omitted at this time. These authors find that certain insects belonging to the orders Hemiptera, Coleoptera, Diptera, and Hymenoptera are carriers of the chestnut-blight organism. Because of the positive evidence secured in the few studies previously made on insects as agencies in the dissemination of plant diseases, it will not be surprising if it is found in future investigations that insects are a very important factor in the dispersal of many plant-pathogenic fungi.

The insects used in these tests were collected in diseased peanut fields near Eutaw, Thomasville, Marion Junction, Greensboro, and Auburn, Ala., and placed in sterile test tubes or flasks plugged with cotton. After being brought into the laboratory, each insect was dropped into a measured amount of water, in case it was desired to determine the number of conidia upon its body. After agitating the tubes vigorously a drop of the wash water was examined under the low-power lens of a microscope, the number of conidia in the drop were counted, and from this the total number of conidia was estimated.

In case fecal discharges were examined, each deposit was macerated in a drop of water on an object slide, and a count was made with the aid of the low-power lens. Because of the presence of undigested bits of plant tissue and the impossibility of one's being sure that no conidia escaped notice and that none were unwittingly counted twice, these determinations can not be exact. They very closely approximate the true number, however, since several counts of the same slide were made and the average taken as the final number.

A total of 75 insects collected in five different counties has been examined in the course of these tests, 54 of which gave positive results. Four orders of insects—namely, Orthoptera, Lepidoptera, Coleoptera, and Hemiptera—were represented among the positive tests. Of the 56 grasshoppers and katydids examined, 38 were found to be bearers of *Cercospora personata*. No attempt has been made to classify these Orthoptera, but several different genera were represented. Of the roasting-ear worms, *Heliothis obsoleta*, which were examined, nine were found to void conidia of *Cercospora* in their feces. Eight members of the Coleoptera were examined, six of which gave positive results. Three of these were lady beetles, *Megilla maculata*; one a blister beetle, *Epicauta vittata*; and the other two were fireflies, *Chauliognathus* sp. A single member of the Hemiptera, one of the leaf hoppers, was examined and found to be a carrier.

<sup>1</sup> Studhalter, R. A., and Ruggles, A. G. Insects as carriers of the chestnut blight fungus. Penn. Dept. Forestry Bul. 22, 33 p., 4 pl. 1915.

Table IV records the results of an examination of 36 of the 75 insects collected. The remainder of the record is not given, since it would add nothing which is not indicated in this tabulated portion.

TABLE IV.—Record of examination of insects for conidia of *Cercospora personata*

No.	Name of insect.	Date of collection.	Locality.	Number of conidia of <i>Cercospora personata</i> .		Other fungi.	Remarks.
				On body.	In feces.		
1	Grasshopper.....	Aug. 10	Eutaw, Ala.	1	8		No note was made of the occurrence of <i>Cercospora personata</i> or other organism in feces.
2	do.....	11	do.....	1			
3	Roasting-ear worm ( <i>Heliothis obsoleta</i> ).	14	do.....	2			
4	Grasshopper.....	16	do.....	4			
5	do.....	16	do.....	1			
6	do.....	16	do.....	5			
7	do.....	26	do.....	4			
8	do.....	26	do.....	6			
9	do.....	26	do.....	3			
10	Firefly ( <i>Chauliognathus</i> sp.).	26	do.....	3			
11	Grasshopper.....	Sept. 6	Auburn, Ala.	1,250		Species of <i>Alternaria</i> , <i>Helminthosporium</i> , and <i>Fusarium</i> .	Seven insects were placed in a flask and within a half hour after their capture they were examined. By agitating them in 25 c. c. of water conidia from the surface of the bodies and from the feces are included.
12	do.....	7	do.....	0	0	2,500 conidia of <i>Helminthosporium Ravenelii</i> .	
13	do.....	8	do.....	10	8	<i>Fusarium</i> , <i>Alternaria</i> .	
14	do.....	8	do.....	0	13	Species of <i>Fusarium</i> and <i>Alternaria</i> .	
15	do.....	8	do.....	0	0		No determination of conidia on bodies was made.
16	do.....	9	do.....	0	18		
17	do.....	9	do.....	0	250		Single fecal discharge.
18	do.....	9	do.....	0	200		
19	Roasting-ear worm ( <i>Heliothis obsoleta</i> ).	9	do.....	0	1,050		
20	Lady beetle ( <i>Megilla maculata</i> ).	9	do.....	8	0		Total number of conidia contained in three fecal discharges. The presence of conidia upon the bodies was not determined.
21	Roasting-ear worm ( <i>Heliothis obsoleta</i> ).	10	do.....	0	50		
22	do.....	10	do.....	0	17		
23	do.....	10	do.....	0	39		
24	do.....	10	do.....	0	27		
25	do.....	10	do.....	0	0		
26	do.....	10	do.....	0	17		
27	do.....	10	do.....	0	28		
28	do.....	10	do.....	0	25		
29	Blister beetle ( <i>Epicauta vittata</i> ).	10	do.....	0	27		Two fecal discharges were examined.

TABLE IV.—Record of examination of insects for conidia of *Cercospora personata*—Continued

No.	Name of insect.	Date of collection.	Locality.	Number of conidia of <i>Cercospora personata</i> .		Other fungi.	Remarks.
				On body.	In feces.		
30	Lady beetle ( <i>Megilla maculata</i> ).	Sept. 20	Auburn, Ala.	9	0	.....	
31	Grasshopper.....	18	.....do.....	0	6	Several hundred conidia of <i>Helminthosporium Ravenelii</i> present.	
32	Katydid.....	18	.....do. . .	0	92		Two discharges.
33	Grasshopper.....	18	.....do... .	0	3	Many <i>Fusarium</i> sp. conidia.	
34	.....do.....	20	....do.. .	0	6	Few <i>Alternaria</i> sp. conidia.	
35	.....do.....	20	....do .	0	0	<i>Puccinia cassipae</i> B. and C., <i>Helminthosporium Ravenelii</i> .	Over 500 spores of each estimated to be present in a single discharge.
36	Leaf hopper.....	20	.....do . .	8	0	.....	

Grasshoppers were found to carry *Cercospora personata* conidia on their bodies and also to void them in their feces. The number of conidia to be found within and upon any individual insect depends naturally upon whether or not it has eaten diseased tissue within a short time prior to its capture. The largest number of conidia of *C. personata* found in a single fecal discharge of a grasshopper brought in from the field was 250.

In order to ascertain whether or not feeding grasshoppers either avoid or select diseased leaf tissue, 13 were brought into the laboratory, where they could be closely observed and given diseased peanut leaves as food. Three of them seemed to prefer leafspot tissue, since they ate little except the affected tissue. The others were indifferent in their choice of food, but seemed not to avoid the diseased spots. The conidia in the discharges of some of these insects were too numerous to count.

Passage through the alimentary canal of grasshoppers does not destroy the power of germination of the conidia of *Cercospora personata*. Conidia which had been voided were found to germinate within 12 to 18 hours when placed in drops of water. In fact, some were found to have already germinated at the time of discharge. When it is realized that these conidia-laden discharges are suitable situations for spore germination and a favorable pabulum for subsequent growth, and that they are commonly deposited upon leaves, it is seen that this is not an impossible means of causing infection. Since grasshoppers, which have notoriously strong powers of flight, were among the insects examined with positive results, they no doubt are potent agencies in the dissemination of leafspot for considerable distances. It is believed that the peculiar results in the tests on rotation and seed disinfection, as well as the correlation between the presence of leafspot and certain temperature and moisture conditions

previously reported, is due in part to the fact that grasshoppers and certain other insects are carriers of the leafspot organism.

It might be interesting to note in this connection that it seems to be generally true that peanut fields in which grass and weeds had been permitted to grow unmolested, as exemplified by fields 4 and 6 in Table I, and which consequently afforded a more attractive feeding ground for grasshoppers, are much more severely attacked by *Cercospora* than those in which good cultivation had been given. Several small fields have also been found upon which chickens and turkeys ranged in which leafspot was doing inappreciable harm, while fields somewhat farther away from the farm buildings were seriously affected. It is believed that the relative freedom from leafspot here observed is to be attributed largely to the destruction of the insects by fowls.

In most cases no attempt was made to determine the presence of other fungi upon the insects taken. Among the other forms noted, however, were *Helminthosporium Ravenelii* B. and C., an organism very abundant upon the inflorescence of *Sporobolus indicus*; *Puccinia cassipis* B. and C., which is parasitic on species of *Ipomoea*, a common weed; and species of *Alternaria* and *Fusarium*. According to an estimate made, a single fecal deposit of a grasshopper contained 2,500 conidia of *Helminthosporium Ravenelii*. A katydid taken at Marion Junction and one at Auburn each voided a vast number of morning-glory rust spores. Insects 21 to 28 (Table IV) indicate the manner in which this form may carry infections for short distances. The blister beetle is another insect which feeds upon peanut plants and which therefore discharges conidia from its alimentary canal. The other species of insects taken appear to carry conidia only upon their bodies. It seems very probable, judging from the evidence at hand, that any insect which feeds upon peanut foliage is a disseminator of leafspot, and that any of them which frequent peanut fields may serve as carriers.

#### SUMMARY

(1) Rotation by itself is not effective under field conditions in eliminating leafspot, as evidenced by a field in which peanuts had not been grown for 11 years and in which 95 per cent of the plants were diseased by August 31, with an estimated loss in yield of 19.5 per cent.

(2) Seed disinfection with copper sulphate or formaldehyde before planting does not prevent leafspot. Shelling peanuts before planting to eliminate the danger of infection from conidia which may have been adhering to the surface of the shell does not prevent the disease. Seed treated in these ways, when planted on land which had previously borne diseased peanuts, produced a crop which was 100 per cent diseased. Seed treated and planted on soil which had borne no peanuts for at least four years previously produced a crop 13 per cent of whose plants were more or less affected with leafspot. Crop rotation, therefore, when combined with seed treatment, will not eliminate leafspot.

(3) An approximation of the total leafspot area involved by *Cercospora personata* showed that the photosynthetic area had been decreased 35.07 per cent. Estimations of decrease in yield of peas of from 5 to 20 per cent as the result of leafspot are therefore regarded as reasonable.

(4) No correlation between the presence of certain conditions of temperature and moisture and the prevalence of leafspot exists, because of the fact that air currents and certain insects are carriers of *Cercospora personata*.

(5) As the result of 210 glycerin exposure-plate tests at Eutaw, Ala., substantiated by a series at Auburn, Ala., it is concluded that *Cercospora personata* is wind borne. Seventy-eight of these 210 exposure plates gave positive results. At no time from August 9 to August 26 was there a period of maximum spore dispersal as revealed by the exposure plates. The maximum number of conidia entrapped on any single plate was four. This does not represent the true condition, since the method used in washing the plates failed to remove all conidia. Rains rendered the air temporarily free from *Cercospora*, and dew prevented the dispersal of conidia at night and in the early morning.

(6) From an examination of 75 insects collected in five localities, of which 54 gave positive results, it is concluded that insects are disseminators of the leafspot fungus. Four orders of insects are included in these positive tests: Orthoptera, represented by grasshoppers and katydids; Lepidoptera, by larvæ of *Heliothis obsoleta*; Coleoptera, by lady beetles, blister beetles, and fireflies; and Hemiptera, by leaf hoppers. Grasshoppers, katydids, roasting-ear worms, and blister beetles eat diseased peanut foliage and void conidia in their fecal discharges. A single deposit from a grasshopper contained 250 conidia of *Cercospora personata*. Another specimen discharged 2,500 conidia of *Helminthosporium Ravenelii* in a single deposit. Grasshoppers may also carry conidia on the surface of their bodies. Leaf hoppers, lady beetles, and fireflies transport conidia on their bodies as a result of having come in contact with diseased leaves. A larva of *Heliothis obsoleta* voided a maximum of 1,050 conidia of *Cercospora personata*. Other fungi, among which are *Puccinia cassipes*, *Alternaria* sp., and *Fusarium* sp., were found in the fecal discharges of grasshoppers and katydids.

(7) Alimentation in insects does not destroy the viability of *Cercospora personata*.

(8) Grasshoppers, because of their powers of flight, are capable of carrying the leafspot organism considerable distances. The ineffectiveness of crop rotation combined with seed treatment to eliminate leafspot from peanut fields is very probably due to the fact that air currents and certain insects are agents in its dissemination.

## RELATION BETWEEN THE PROPERTIES OF HARDNESS AND TOUGHNESS OF ROAD-BUILDING ROCK

By PRÉVOST HUBBARD, *Chemical Engineer*, and F. H. JACKSON, JR., *Assistant Testing Engineer, Office of Public Roads and Rural Engineering*

It has for some time past become increasingly evident to engineers interested in the testing of road materials that from the standpoint of the road builder some of the most important physical properties of rock are not independent, but are more or less definitely related to each other. In 1913, Mr. L. W. Page,<sup>1</sup> Director of the United States Office of Public Roads and Rural Engineering, called attention to some of these points, and suggested that, as the volume of data relating to the subject became greater, it might be possible to determine the dependent variable by reference to suitable curves showing the relative values of tests for thousands of individual cases, and thus dispense with one or more of the tests now in use. The large amount of additional data which have accumulated since that time makes it possible to take up the subject again, with a view to determining just what physical tests are necessary in order to judge properly the fitness of a rock for use in road construction.

It is now generally recognized that any stone, to be suitable for use in macadam construction, must possess to a certain degree, depending on circumstances such as character of traffic and method of construction, three distinct physical properties, which may be briefly defined as follows:

- (1) Hardness, the resistance which a rock offers to the displacement of its surface particles by abrasion;
- (2) Toughness, the resistance which a rock offers to fracture under impact;
- (3) Binding power, the ability which the dust from the rock possesses, or develops by contact with water, of binding the larger rock fragments together.

Of these, the first two are of particular interest from the standpoint of the present discussion, and they may be very briefly described as follows:

The degree of hardness of a rock is determined by what is known as the Dorry method. It consists essentially of subjecting a cylinder, 25 mm. in diameter, of the material to be tested, to the abrasive action of crushed quartz sand fed upon a revolving steel disk, against which the test

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<sup>1</sup> Page, L. W. Relation between the tests for the wearing qualities of road-building rocks. *In Amer. Soc. Testing Materials, Proc. 16th Ann. Meeting, 1913, v. 13, p. 983-992, 7 fig., 1913. Discussion, p. 993-995.*

— Tests of materials used in the construction of macadamised roads. *Permanent Internat. Assoc. Road Cong., 3d Cong. London, 1913, Rpt. 76, 27 p., 15 fig. 1913.*

specimen rests. The end of the specimen is ground away in inverse ratio to its hardness, so that the hardness may be computed by determining the loss in weight after any given number of revolutions of the disk. The coefficient of hardness discussed later is obtained by subtracting one-third of the loss in weight in grams from 20, after 1,000 revolutions of the disk.

The degree of toughness is determined by the Page impact method. A cylinder 1 inch in diameter and 1 inch high, cut from the rock specimen, is subjected to the impact caused by the free fall of a 2-kgm. weight dropped from successively increasing heights until the energy of the blow is sufficient to fracture the test specimen. The test consists of a 1-cm. fall for the first blow, followed by falls increased by 1 cm. after each blow until failure occurs. The height from which the weight drops when failure takes place is used as a measure of the toughness of the material.

Since the establishment of the Road-Material Laboratory by the United States Government, upwards of 3,000 samples, representing every known variety of road-building rock, and obtained from every State in the Union, as well as from foreign countries, have been subjected to the tests outlined above. The results of these tests are plotted in graphic form in figure 1. The coefficients of hardness are plotted as abscissæ and the factors of toughness as ordinates. Each small circle represents the corresponding hardness and toughness of an individual rock sample. The large circles represent the average of all the coefficients of hardness for each value of toughness. Hardness values range from 0 to 20 and toughness values from 1 to 47.

A study of this curve brings out the following points:

- (1) That the average toughness for all tests made is about 9.
- (2) That the average hardness increases with toughness, and that the rate of increase becomes less as the toughness values become larger.
- (3) That individual values of hardness vary through wide limits for low values of toughness, and that the variations from the average decrease uniformly with the increase in toughness up to a certain point, about 20, after which they remain constant with very little variation from the average.
- (4) That, when any given value for toughness falls within certain limits, which define the suitability of the material for macadam-road construction under different traffic conditions, the corresponding value for hardness will fall within similar limits for hardness.

The first three facts are clearly indicated, but in order to substantiate the last deduction it will be necessary to define the limiting values of hardness and toughness which experience has shown should be applied when judging the fitness of stone for use in macadam construction under different traffic conditions. Such limiting values for toughness are shown on the curve in the ordinates at 4.5, 9.5, and 18.5, and the

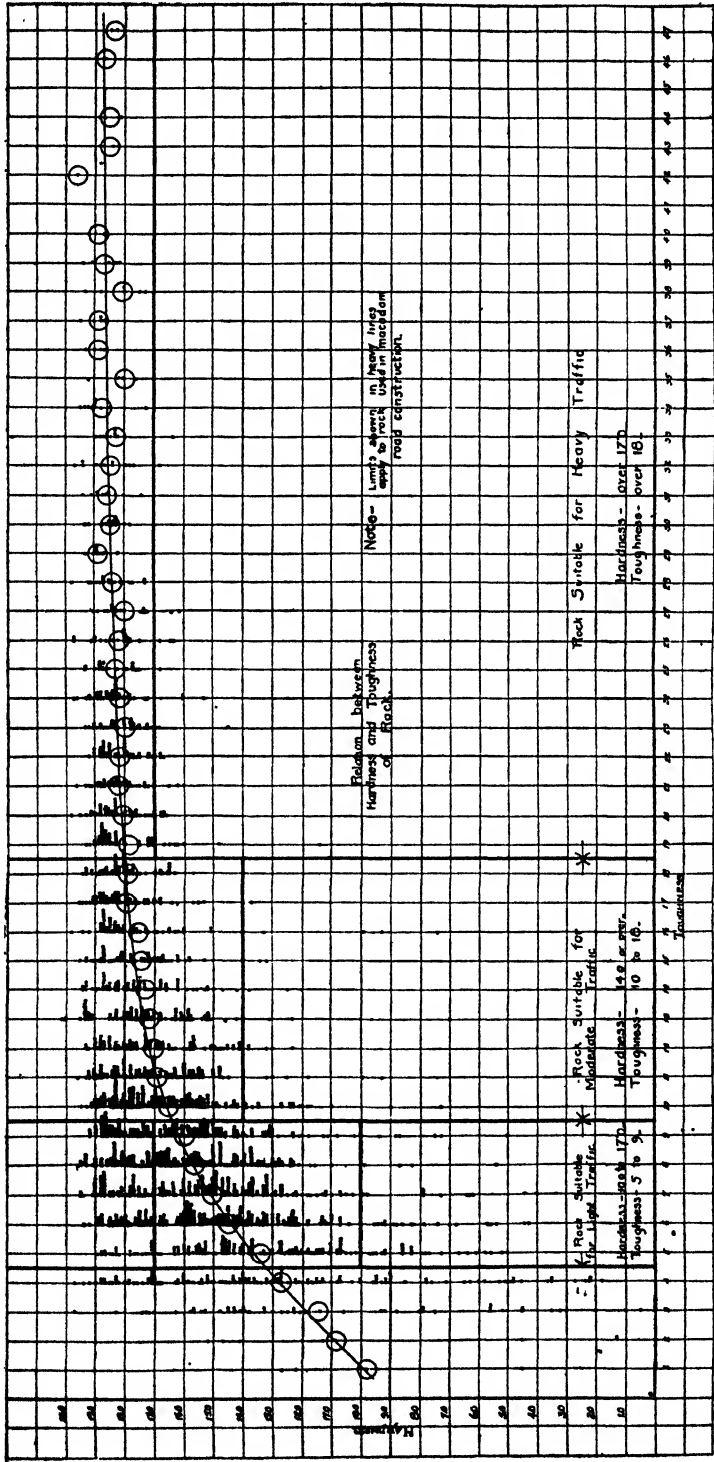


FIG. 1.—Curve showing the results of tests of about 3,000 samples of road-building rock.



corresponding limiting values for hardness at 10, 14, and 17. In other words, after making all allowances for variations due to local conditions, it may be fairly assumed that stone for use under light, horse-drawn, steel-tired vehicles should show a toughness of from 5 to 9 and a hardness of from 10 to 17; for moderate traffic a toughness of from 10 to 18 and a hardness of over 14, and for heavy traffic a toughness of 19 or over and a hardness of 17 or over. The terms "light," "moderate," and "heavy" in this connection refer to the total volume of traffic upon the road, calling, say, under 100 teams a day "light," 100 to 250 "moderate," and over 250 "heavy."

Practically all the values of hardness shown in figure 1 are above the various lower limits set by the best water-bound macadam-road practice.

For light-traffic conditions, 94 per cent of all the samples tested have a hardness of more than 10; for moderate traffic, 95 per cent have a hardness of more than 14; and for heavy traffic, 94 per cent have a hardness of more than 17.

In other words, if it be assumed that the curve (fig. 1) represents a fair average of all available types of road-building rock, it would seem that a determination of the toughness of any particular sample of rock shows, for all practical purposes at least, whether it is hard enough to be satisfactorily used in construction.

If the curve be referred to again, it will be seen that a large number of hardness tests appear above the upper limit of 17 set for light-traffic conditions. Although on its face this would indicate that a determination of the hardness is necessary in this instance, reference to test records show that by far the greatest number of these tests (about 75 per cent) are on granites, quartzites, and hard sandstones, which are unsuited for use in the wearing course of water-bound macadam roads, owing to their lack of binding power, as shown by actual test.

Finally, the results of 2,500 individual routine tests made by the Office of Public Roads and Rural Engineering show that for practical routine work the hardness test adds nothing to our knowledge of the value of any particular rock sample for use in water-bound macadam-road construction over that obtained from the toughness test.

While the binding or cementing value of a rock is a most important consideration from the standpoint of ordinary macadam construction, the same is not true of broken-stone roads which are surface treated or constructed with an adhesive bituminous material. The hardness of the rock is also of relatively less importance, owing to the fact that the fine mineral particles produced by the abrasion of traffic combine or should combine with the bituminous material to form a mastic which is held in place and protects the underlying rock from abrasion so long as by proper maintenance it is kept intact. The toughness of the rock, however, is of more importance, as the shock of impact is to a considerable extent transmitted through the seal coat and may cause the underlying fragments

to shatter. It would therefore seem that the minimum toughness of a rock for use in the construction of a bituminous broken-stone road or a broken-stone road with a bituminous-mat surface should for light traffic be no less than for ordinary macadam subjected to the same class of traffic. For moderate and heavy traffic, however, the same minimum toughness may probably prove sufficient, owing to the cushioning effect of the bituminous matrix. No maximum limit of toughness need, however, be considered for any traffic.

In the case of bituminous concrete roads, where the broken stone and bituminous material are mixed prior to laying and consolidation, it would perhaps appear advisable to set a minimum toughness of 6 or 7 for light-traffic roads instead of 5, in order to insure against the possibility of the fragments of rock which have been coated with bitumen being fractured under the roller during consolidation, and of 12 or 13 for moderate and heavy traffic, instead 10 and 19, as in the case of water-bound macadam roads.

For broken-stone roads which are to be maintained with dust palliatives, it would appear that the same limits of toughness should hold as for ordinary macadam.

For easy reference the following limits of toughness are given in Table I, as representing facts developed in the foregoing discussion. It is, of course, quite probable that these limits will require modification as the correlation of laboratory tests to service results becomes more perfect.

TABLE I.—Limits for toughness for rock used in the construction of broken-stone roads

Type of road.	Light traffic.		Moderate traffic.		Heavy traffic.	
	Mini-mum.	Maxi-mum.	Mini-mum.	Maxi-mum.	Mini-mum.	Maxi-mum.
Macadam.....	5	9	10	18	19	.....
Macadam with dust palliative.....						
Macadam with bituminous mat .....						
Bituminous broken stone with seal coat. ....	5	.....	10	.....	10	.....
Bituminous concrete with or without seal coat. ....						
	7	.....	13	.....	13	.....

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### NITROGEN CONTENT OF THE HUMUS OF ARID SOILS<sup>1</sup>

By FREDERICK J. ALWAY, *Chief, Division of Soils, Agricultural Experiment Station of the University of Minnesota*, and EARL S. BISHOP, *Industrial Fellow, Mellon Institute*

#### HISTORICAL REVIEW

One of the most generally recognized characteristics of arid soils (16, p. 163; 15, p. 415; 17, p. 72; 13, p. 147)<sup>2</sup> is the high content of nitrogen contained in their humus, the *matière noire* of Grandeau (6, p. 148).

Attention was first called to this by Hilgard and Jaffa (10), who stated (p. 69):

It thus appears that on the average the humus of the arid soils contains three times as much nitrogen as that of the humid; that in extreme cases the difference goes as high as 6 to 1.

It is somewhat remarkable that so few other investigators have made any attempt to test this generalization in the case of the soils from the arid portions of either this or any of the other continents.

Fulmer (5) determined the humus nitrogen in 53 soils from Washington, a State with winter rains and summer droughts. In the case of two soils from Skagit County, which has an annual precipitation of about 46 inches, he found 10.46 and 12.04 per cent, respectively, of nitrogen in the humus.

Nabokich (14, p. 339) reports six samples from Bessarabia with from 11.1 to 18.9 per cent of nitrogen in the humus. It seems probable, however, that he has confused the use of the term "humus" as employed on the continent of Europe (organic matter of the soil as determined by combustion with copper oxid) with the sense in which it is generally used in this country. However, he makes a direct comparison of the Danubian soils with those of California, as follows:

In contrast with the soils of the dry steppes of southern Russia, the humus of the borders of the Danube is quite as rich in nitrogen as that of the soils of the steppes of California and Transcaucasia. The alluviums of the Danube are even richer than those of the Arax.<sup>3</sup>

<sup>1</sup> The work reported in this paper was carried out in 1911 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Assistant in Chemistry.

<sup>2</sup> Reference is made by number to "Literature cited," p. 915-916.

<sup>3</sup> Author's translation (14, p. 339).

Southern Bessarabia has an annual precipitation of about 15 inches, most of it falling during the growing season. Such a climate in this country is commonly referred to as "semiarid."

Loughridge (12, p. 87), in a comprehensive study of the distribution of humus in California soils to a depth of 12 feet, made nearly 1,000 determinations of humus nitrogen, stating that—

of these there were about 64 where the humus was found to contain more than 10 per cent nitrogen, fourteen of these had from 15 to 20 per cent and but five had more than 20 per cent. . . The general average for all the soils, including the marsh lands, is 5.92 per cent for the first foot, 5.60 per cent for the upper three feet and 5.57 per cent for the entire depth of twelve feet.

Our work was an outgrowth of previous investigations in the same laboratory of the humus of semiarid soils. Samples from the semiarid prairies of Canada had been found by Alway and Trumbull (3) and Alway and Vail (4) to show percentages of nitrogen in the humus similar to those in soils from humid regions. In subsequent, as yet unpublished, studies by Alway and Trumbull and by ourselves many surface soils, representing various soil types and the different degrees of aridity found in Nebraska as well as many samples from the semiarid and desert portions of New Mexico and Arizona, were analyzed without finding even one in which the humus contained as much as 10 per cent of nitrogen. The question then naturally arose as to whether we would meet with similar results if we worked with arid soils from a region of winter rains and summer droughts. Having available a small collection of samples of California soils personally collected in 1909 by one of us in connection with another study, we subjected these to analysis. As our analyses were not fully confirmatory of Hilgard's conclusions, we delayed publication of the results, hoping to be able to continue the work with a more extensive series of samples from California. Since then, Loughridge (12) has reported his findings, with which ours are in general agreement. The question as to the conditions under which a high content of nitrogen in the humus is found in arid soils does not appear as yet at all satisfactorily answered. We present our data in the hope that some one more conveniently located for the collection of the necessary samples will take up the study.

The data upon which Hilgard's conclusions were based are given in the Annual Reports of the Agricultural Experiment Station of the University of California from 1884 to 1902. The method used for the determination of humus nitrogen is described by Hilgard (7, p. 247) and Jaffa (11, p. 35): "Two portions of 5 or 10 grams of air-dried soil (depending on richness in humus)" were placed in prepared filters, washed first with dilute (0.5 to 1.0 per cent) hydrochloric acid, until the filtrate gave no reaction for lime and magnesia, and then with distilled water to neutral reaction. Then the one portion was washed with repeated portions of 6 to 7 per cent ammonia solution until the washings became colorless while the other

was similarly treated with a 4 per cent potassium-hydroxid or a 3 per cent sodium-hydroxid solution. The ammonia solution was used for the determination of the humus, while in the other the humus nitrogen was determined by the Kjeldahl method. On the assumption that the same compounds had been dissolved by the two solvents, the percentage of nitrogen in the humus was calculated.

While Hilgard's conclusions were based upon determinations in which the humus was extracted with an alkaline hydroxid solution, he later suggested as an alternative the use of the ammonia solution (8, p. 22), this being concentrated and then mixed with magnesia and boiled before being subjected to the Kjeldahl determination.

The correctness of the assumption that the ammonia solution dissolves the same compounds or the same proportions of the total nitrogen as the alkaline hydroxids is open to serious question. A mere determination of the nitrogen removed by the two solvents does not suffice to decide the question. The ammonia is likely to combine with some of the dissolved organic matter of the soil, with the result that after the concentration of the extract, preliminary to the Kjeldahl digestion, there may still be present some nitrogen derived from the ammonia in addition to that extracted from the soil. The attempt to eliminate any such combined nitrogen by digestion with magnesia previous to the Kjeldahl determination is unsatisfactory, as the magnesia may decompose some of the nitrogen compounds extracted from the soil with the elimination of ammonia. A determination of the organic carbon in both solvents should be made, and if this is not the same the nitrogen in the alkaline hydroxid solution is not to be regarded as that corresponding to the whole of the organic matter dissolved by the ammonia.

#### EXPERIMENTAL WORK

We have confirmed Hilgard and Jaffa's (10) observation that after prolonged extraction of a soil with either ammonia or alkaline hydroxid solution the other fails to extract any appreciable amount of black material. Using 10-gm. portions of both a semiarid and a humid soil, we treated with a 4 per cent ammonia solution until the washings became colorless, placed the residues together with 500 c. c. of alkaline hydroxid solution in stoppered bottles, shook these at frequent intervals for eight hours, and then allowed them to stand overnight. In the case of potassium hydroxid, we tried concentrations of 64, 32, 16, 8, 4, 2 per cent and of sodium hydroxid of 36, 18, 9, 4.5, 2.25 per cent. In all cases the amount of coloring matter extracted was so small that the humus could not be satisfactorily determined even by the delicate photometric method (2). Accordingly, it seems safe to assume that the ammonia solution removes the dark coloring matter as completely as the alkaline hydroxids. However, there appears no reason for assuming that a definite relation exists between the quantity of this pigment and the

amount of ammonia-soluble matter in a soil. Comparisons of the color of the ammonia extracts with their content of dissolved matter show that this relation is variable for different depths in the same field and for the same depth in different localities (2, p. 13).

The large number of soils referred to above were analyzed, using the ammonia extract and magnesia, without finding any in which the humus contained as much as 10 per cent of nitrogen. A later critical study of the method showed that the results were not reliable, the amount of humus nitrogen found being affected by the extent to which the solution was concentrated before adding magnesia and also by the time of digestion with the latter. One result of this was that, while parallel determinations gave concordant results, those run one after the other, using the same ammonia solution, gave widely varying results.

The extraction of the humus by the Hilgard-Jaffa method (10) in the case of many soils, especially those of very fine texture, is extremely tedious, being in this respect similar to the Hilgard method for the determination of humus, for which in the case of some soils 10 days or even longer is necessary (7, p. 320). For this reason we sought to devise a more expeditious and convenient method. Using two representative soils, one a silt loam from the Nebraska Experiment Station farm containing 2.41 per cent of humus and 0.245 per cent of total nitrogen, and the other a clay loam from Indian Head, Saskatchewan, Canada, with 1.56 per cent of humus and 0.248 per cent of total nitrogen, we tried shaking 10 gm. of dry soil with 500 c. c. of a 4 per cent potassium-hydroxid solution for periods of 0.5, 1, 2.5, 5, 9, 12, and 24 days. During the working portion of the day the glass-stoppered bottles containing the mixtures were shaken at intervals of about one hour. With both soils the amount of nitrogen dissolved ceased to increase at the end of nine days. Repeated extraction of the same soil with fresh alkali solution, which might have given different results, was not tried.

This method was then compared with that of Hilgard and Jaffa (10), using in the case of five arid soils from California (Table I) both a 4 per cent potassium and a 6 per cent sodium-hydroxid solution.

TABLE I.—Comparison of methods for the determination of humus nitrogen

Determination.	Humus nitrogen.			Total nitrogen.
	New method.	Hilgard-Jaffa method.		
		With potas- sium hy- droxid.	With sodium hydroxid.	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
A. First.....	0.162	0.150	0.176	0.260
Second.....	.159	.152	.180	
Third.....		.153	.169	
Average.....	.160	.152	.175	
D. First.....	.023	.021	.025	.031
Second.....	.020	.020	.018	
Third.....		.020	.023	
Average.....	.021	.020	.022	
F. First.....	.024	.023	.026	.032
Second.....	.025	.021	.026	
Third.....		.020		
Average.....	.025	.021	.026	
I. First.....	.058	.045	.037	.104
Second.....	.064	.046	.038	
Third.....		.049	.041	
Average.....	.061	.047	.039	
L. First.....	.047	.034	.030	.070
Second.....	.047	.035	.035	
Third.....		.038	.035	
Average. . .	.047	.036	.033	

The results are only fairly concordant, but the extraction of nitrogen was as complete as by the Hilgard-Jaffa method, and for our study this was the most important consideration.

Using this method, employing a 4 per cent potassium-hydroxid solution and shaking at intervals for 9 days, we determined the humus nitrogen in 16 samples of arid soils from California (Table II). The humus was determined by the Hilgard method (1, p. 319). Duplicate and, in most cases, triplicate determinations were made of both the total nitrogen and the humus nitrogen, and duplicate determinations of the humus.



TABLE II.—*Relation of nitrogen to humus in arid soils from California*

Sample No.	Depth.	Location and description of soil.	Humus.	Humus ash.	Total nitrogen.	Humus nitrogen.	Nitrogen in humus.	
							Found.	Maximum possible.
	<i>Inches.</i>		<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
A.....	0-3	Berkeley. Adobe, virgin.	1.71	0.46	0.260	0.160	9.3	15.2
B.....	0-3	do.....	1.19	.25	.233	.119	10.0	19.6
C.....	0-6	Waterford. Alluvium, cultivated.	.62	.39	.054	.035	5.6	8.7
D.....	0-6	Ceres. Loam, cultivated.	.31	.25	.031	.021	6.7	10.0
E.....	0-3	Fresno. Red hog-wallow land, virgin.	.47	.52	.026	.019	4.1	5.5
F.....	0-6	Clovis. Red land, cultivated.	.29	.18	.032	.025	8.3	11.0
G.....	0-6	Fresno. Black adobe, cultivated.	1.39	.92	.144	.087	6.3	10.4
H.....	0-5	Fresno. Dry bog, virgin.	.75	.45	.078	.030	4.0	10.4
I.....	0-5	Fresno. Dry bog, cultivated.	.84	.29	.104	.061	7.2	12.4
J.....	0-5	Clovis. Red land, cultivated.	.38	.16	.060	.032	8.8	15.6
K.....	0-5	do.....	.36	.17	.052	.037	10.4	14.4
L.....	0-6	Delano. Alluvium, cultivated.	.40	.14	.070	.047	11.8	17.5
M.....	0-6	Delano. Red land, cultivated.	.50	.32	.061	.036	7.5	12.2
N.....	0-6	do.....	.38	.17	.061	.034	9.0	15.1
O.....	0-2	Delano. Red land, virgin.	1.00	.34	.159	.115	11.5	15.9
P.....	0-2	Delano. Alluvium, virgin.	1.17	.20	.187	.140	12.0	16.0
Average.....			.73	.....	.101	.062	8.3	13.1

All the samples, except the two from Berkeley, Cal., were secured in the San Joaquin Valley in the vicinity of Modesto, Fresno, and Delano, where the normal annual precipitation amounts to 10.9, 9.0, and 6.1 inches, respectively. Samples A and B were both taken from the high hill just east of the buildings on the grounds of the University of California. Sample A is a composite of 20 samples from near the summit, and B of the same number from the lighter colored soil to the west, below the summit. Sample C was from a cultivated field east of Modesto and 4 miles west of Waterford. Sample D was from a fallowed field 3 miles south of Hickman and 12 miles east of Ceres, E from the virgin red hog-wallow land 7 miles north of Fresno, and F from the red lands 10 miles east of Clovis. The last-named had been under cultivation from 15 to 20 years. Sample G is a black adobe from the same farm as sample F, and the field had been under crop for about 7 years. Samples H and I are "dry-bog soils" from a hilltop near the farm from

which F and G were secured. H was from virgin soil, while I was from land which had been formerly cultivated, but allowed to revert to grass about 10 years before. Samples J and K were taken from two fallowed fields of red land about 2 miles east of Clovis. The remaining samples were from near Delano—L, from a field under cultivation for 15 years and M and N from fallows on red land north of the White River.

Of the 16 samples only 5 show as high as 10 per cent of nitrogen in the humus. For the 6 samples of virgin soil the average is 8.5 per cent, with a maximum of 12.0 and a minimum of 4.0 per cent. For the 10 of cultivated soils the corresponding data are 8.1, 11.8, and 5.6 per cent, respectively. The maximum possible percentages of nitrogen in the humus—the relation of the total nitrogen to the humus—ranged from 5.5 to 19.6 per cent, with an average of 13.1. Hilgard (9, p. 424), in a comparison of the average composition of 313 arid and 466 humid soils, reports the former to show 0.75 per cent humus and 15.87 per cent of nitrogen and the latter 2.70 per cent of humus, with only 5.45 per cent of nitrogen.

There is no reason to doubt the reliability of the humus determinations upon which Hilgard's generalizations are based. A careful study (1) has shown that his method, as carried out by himself, gives results strictly comparable with those of the Moores-Hampton method. We have examined the original data on the humus determinations by Hilgard and his assistants and in only a very few cases do we find a humus-ash content sufficiently high to make the determination appear inaccurate. These percentages of humus ash, while not reported in the tables in Hilgard's articles discussing the relation of the nitrogen content of humus to climate, may be found in the original reports referred to above.

In that we found 5 out of 16 arid soils to have over 10 per cent of nitrogen in the humus after having failed to find any humid or semiarid soil with such a high percentage, our study tends to confirm the work of Hilgard that high percentages are to be found in the arid but not in the humid soils. This high nitrogen content of the humus, however, does not appear so general in the arid soils as to serve as an at all reliable means of identification.

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# LIFE-HISTORY STUDIES OF THE COLORADO POTATO BEETLE

By PAULINE M. JOHNSON, *Scientific Assistant*, and ANITA M. BALLINGER, *formerly Preparator, Truck-Crop and Stored-Product Insect Investigations, Bureau of Entomology*.

## INTRODUCTION

The experiments on the life history of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the details of which follow, were suggested by Dr. F. H. Chittenden, in charge of Truck-Crop and Stored-Product Insect Investigations of the Bureau of Entomology, and were conducted under his direction. These studies were necessarily carried on indoors for the most part and under somewhat unnatural conditions. Had they been conducted out of doors, the probabilities are that in any well-kept field of potatoes (*Solanum tuberosum*) the beetles would have passed through a period of estivation; and if the potatoes had been grown under weedy conditions, where the beetles had access to wild solanaceous plants, the third generation would have been produced. All experiments were performed in the District of Columbia during the season of 1914. The temperature during the period of the work was exceedingly high, with more than the normal rate of humidity.

## GENERATION EXPERIMENTS

The overwintered beetles of this species made their first appearance after hibernation on April 29 on *Solanum jasminoides*, an ornamental plant growing in the insectary garden. Beetles were collected and pairs isolated in jars for experimental purposes. After feeding for a few days the females began depositing their characteristic orange-colored eggs (Pl. LXIII, fig. 1) in masses on the underside of the leaves near the tips. The egg masses averaged from 35 to 45 eggs each, except in two cases observed, in which as many as 70 and 72 eggs, respectively, were counted. When the potato plants first emerged from the ground, the beetles showed a decided preference for them, deserting the foliage of *S. jasminoides* for the more tender leaves of the potato.

The fecundity of single females, under the conditions described, is shown in Tables I to VIII.

## FIRST GENERATION

TABLE I.—Eggs produced by a single overwintered female of the Colorado potato beetle; male and female taken in copula on April 30, 1914, and placed in rearing jar with growing potato plant<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 4....	17	17	May 15...	24	24	May 25...	4	4
5....	0	0	16...	0	0	26...	0	0
6....	0	0	17...	0	0	27...	0	0
7....	0	0	18...	16	16	28...	0	0
8....	43	43	19...	0	0	29...	14	14
9....	0	0	20...	0	0	30...	16	16
10....	67	67	21...	10	10	31...	24	1,5,10,8
11....	31	31	22...	9	9	June 1...	3	3
12....	45	45	23...	0	0	Total..	379	.....
13....	28	28	24...	10	10			
14....	18	18						

<sup>1</sup> The male in this experiment died on June 10, the female on June 14.TABLE II.—Eggs produced by a single overwintered female of the Colorado potato beetle; pair collected at College Park, Md., and placed in rearing jar on May 11, 1914, with growing potato plant<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	78	32, 46	May 31...	70	21, 18, 31	June 19...	0	0
12...	51	31, 20	June 1...	25	25	20...	5	5
13...	31	31	2...	21	21	21...	0	0
14...	0	0	3...	0	0	22...	0	0
15...	0	0	4...	2	2	23...	9	9
16...	0	0	5...	0	0	24...	40	40
17...	90	36, 54	6...	0	0	25...	14	14
18...	0	0	7...	0	0	26...	0	0
19...	36	36	8...	0	0	27...	0	0
20...	32	32	9...	0	0	28...	0	0
21...	34	9, 11, 17	10...	6	0	29...	0	0
22...	0	0	11...	0	0	30...	0	0
23...	0	0	12...	0	0	July 1...	0	0
24...	34	34	13...	0	0	2...	0	0
25...	72	24, 48	14...	0	0	3...	0	0
26...	29	20, 9	15...	34	34	4...	0	0
27...	25	25	16...	0	0	5...	24	24
28...	47	47	17...	71	52, 19	Total..	994	.....
29...	33	33	18...	50	50			
30...	28	28						

<sup>1</sup> The male died on June 7, the female on July 7.

TABLE III.—Eggs produced by a single overwintered female of the Colorado potato beetle; male and female collected at College Park, Md., and placed in confinement on May 10, 1914, with growing potato plant<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	44	44	May 26...	0	0	June 9...	0	0
12...	16	16	27...	0	0	10...	0	0
13...	0	0	28...	0	0	11...	0	0
14...	0	0	29...	25	25	12...	0	0
15...	46	46	30...	0	0	13...	0	0
16...	0	0	31...	0	0	14...	23	23
17...	39	39	June 1...	23	23	15...	42	42
18...	0	0	2...	16	16	16...	33	33
19...	36	19, 17	3...	0	0	17...	0	0
20...	11	11	4...	0	0	18...	0	0
21...	0	0	5...	0	0	19...	0	0
22...	0	0	6...	0	0	20...	16	16
23...	0	0	7...	10	10	Total.	389	.....
24...	0	0	8...	0	0			
25...	0	0						

<sup>1</sup> The male died on June 23, the female on September 2.

TABLE IV.—Eggs produced by a single overwintered female of the Colorado potato beetle; pair of adults taken in copulation and isolated in a rearing jar on May 11, 1914, with a growing potato plant<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 11...	57	57	June 4...	56	20, 36	June 28...	0	0
12...	15	15	5...	41	41	29...	0	0
13...	15	15	6...	41	41	30...	33	33
14...	58	58	7...	45	45	July 1...	42	42
15...	0	0	8...	35	35	2...	0	0
16...	54	54	9...	60	60	3...	43	43
17...	57	57	10...	43	43	5...	35	35
18...	0	0	11...	57	57	6...	0	0
19...	33	33	12...	84	19, 65	7...	47	47
20...	25	25	13...	47	47	8...	61	22, 39
21...	21	21	14...	54	54	9...	13	13
22...	31	31	15...	55	55	10...	30	30
23...	34	34	16...	51	51	11...	0	0
24...	0	0	17...	39	39	12...	20	20
25...	56	56	18...	46	34, 12	13...	0	0
26...	26	26	19...	31	31	14...	0	0
27...	27	27	20...	0	0	15...	13	13
28...	0	0	21...	7	7	16...	8	8
29...	37	37	22...	1	1	17...	16	16
30...	42	42	23...	0	0	18...	0	0
31...	30	30	24...	0	0	19...	8	8
June 1...	25	25	25...	0	0	20...	14	14
2...	36	36	26...	0	0	Total.	1,879	.....
3...	0	0	27...	0	0			

<sup>1</sup> The male died on August 1, the female on August 20. In this experiment the duration of egg-laying extended over a period of 70 days, or 10 weeks.

TABLE V.—Eggs produced by a single overwintered female of the Colorado potato beetle; male and female in copula isolated on May 11, 1914, with growing potato in rearing jar<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
May 14..	36	36	June 3..	0	0	June 23..	6	6
15..	0	0	4..	39	39	24..	0	0
16..	43	43	5..	45	14, 31	25..	16	16
17..	0	0	6..	43	43	26..	0	0
18..	20	20	7..	35	35	27..	11	11
19..	0	0	8..	46	33, 13	28..	0	0
20..	0	0	9..	54	54	29..	38	38
21..	54	27, 27	10..	63	34, 11, 18	30..	0	0
22..	0	0	11..	62	30, 32	July 1..	0	0
23..	32	32	12..	24	24	2..	20	20
24..	33	33	13..	57	57	3..	8	8
25..	19	19	14..	34	34	4..	0	0
26..	25	25	15..	33	33	5..	0	0
27..	67	33, 34	16..	31	31	6..	0	0
28..	0	0	17..	34	34	7..	0	0
29..	40	40	18..	25	25	8..	4	4
30..	42	42	19..	0	0	Total..	1, 301	.....
31..	48	12, 12, 24	20..	8	8			
June 1..	32	32	21..	12	12			
2..	43	43	22..	19	19			

<sup>1</sup> The male died on June 25, the female on July 27. Temperatures: Maximum, 98° F.; minimum, 43° average, 72°.

Eggs which were deposited on May 4 hatched on May 12, and the larvæ (Pl. LXIII, fig. 2) fed ravenously until May 28, when they entered the ground to a depth of about 3 inches and transformed to pupæ on May 30. Adults emerged on June 9.

Eggs which were deposited on May 7 hatched on May 16. The larvæ became full grown, pupated on May 31, and entered the soil, from which the adults issued on June 10.

#### SECOND GENERATION

After the adults of the first generation had issued from the ground, three pairs were isolated while in copulation and placed in jars with potato leaves as food on June 17, 18, and 19, respectively.

TABLE VI.—Record of egg deposition of first-generation female of pair 1 of the Colorado potato beetle, confined in rearing jar on June 17, 1914, and fed upon potato foliage<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
June 22..	22	22	July 5...	11	11	July 17...	47	47
23..	45	45	6...	0	0	18...	44	44
24..	11	11	7...	19	19	19...	45	45
25..	23	23	8...	24	24	20...	0	0
26..	0	0	9...	0	0	21...	0	0
27..	0	0	10...	0	0	22...	37	37
28..	44	44	11...	0	0	23...	0	0
29..	2	2	12...	0	0	24...	0	0
30..	15	15	13...	22	22	25...	0	0
July 1..	47	47	14...	0	0	26...	17	17
2..	27	27	15...	0	0	27...	11	11
3..	0	0	16...	0	0	Total..	513	.....
4..	0	0						

<sup>1</sup> The male in this experiment died on June 20, the female on August 4.

The male and female of pair 2, having been confined to the rearing jar on June 18, 1914, fed for a few days upon the potato foliage, after which they entered the ground for hibernation, the female depositing no eggs.

TABLE VII.—Record of egg deposition of first-generation female of pair 3 of the Colorado potato beetle, confined in rearing jar on June 19, 1914, and fed upon potato foliage<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.	Date.	Number of eggs laid.	Number of eggs to a mass.
July 1...	4	4	July 10...	0	0	July 18...	38	38
2...	0	0	11...	0	0	19...	18	18
3...	32	32	12...	0	0	20...	38	38
4...	30	30	13...	0	0	21...	0	0
5...	38	38	14...	0	0	22...	15	15
6...	48	48	15...	0	0	23...	65	65
7...	46	46	16...	0	0	Total..	502	.....
8...	33	33	17...	59	22, 37			
9...	38	38						

<sup>1</sup> The male of this pair went into hibernation on July 20, the female on July 27. Temperatures: Maximum, 102° F.; minimum, 58°; average, 72°.

A mass of eggs which was deposited on June 30 by the female of pair 1 hatched on July 7. The larvæ became full-grown on July 23, pupated on July 25, and emerged as adults on July 31. Another mass of eggs laid on July 10 by the same female hatched on July 16, the larvæ pupating on August 5 and issuing as adults on August 11.



## THIRD GENERATION

When the adults of the second generation had emerged, pairs were isolated as in previous experiments.

TABLE VIII.—Record of egg deposition of second-generation female of a pair of the Colorado potato beetle, confined in rearing jar and fed upon potato foliage<sup>1</sup>

Date.	Number of eggs laid.	Number of eggs to a mass.
1914.		
August 20.....	19	19
21.....	48	48
22.....	0	0
23.....	45	45
Total.....	112	.....

<sup>1</sup> Temperatures: Maximum, 96° F.; minimum, 46°; average, 70°.

In the rearing experiments with the third generation the females of the second generation did not all oviposit. Four pairs began hibernation after feeding for several days. One mass of eggs deposited on August 4 hatched on August 9, the larvæ pupating on August 23 and the adults emerging on August 31. Another egg mass, which was deposited on August 21, hatched on August 26, and the larvæ, becoming full-grown on September 14, entered the ground for pupation, the adults emerging on September 23.

All of the beetles of this third generation were very active and fed voraciously on the foliage of the potato up to September 15.

## LENGTH OF STAGES

Table IX shows the maximum and minimum number of days covered by each of the immature stages in each of the three generations, as obtained from the foregoing rearing experiments.

TABLE IX.—Maximum and minimum length (in days) of immature stages of the Colorado potato beetle in each of the three generations

Generation.	Egg stage.		Larval stage.		Pupal stage.		Total developmental period.	
	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.
First.....	7	9	15	18	10	10	30	37
Second.....	6	7	16	18	6	8	32	41
Third.....	5	5	14	19	8	9	27	35

## NUMBER OF MOLTS AND DURATION OF INSTARS

Eggs of the Colorado potato beetle were segregated and watched carefully to determine the number of molts of the larvæ and the time spent in each instar. It was found that every larva has three molts, with an average of about three days for each instar. Tables X and XI show the dates and number of days required for the molts.

TABLE X.—Number of molts and dates of molting of Colorado potato-beetle larvæ in 1914

Experiment No.	Egg hatched.	First molt.	Second molt.	Third molt.
1.....	July 26	July 29	Aug. 2	Aug. 5
2.....	do.....	do.....	Aug. 1	Aug. 3
3.....	July 30	Aug. 2	Aug. 4	Aug. 8
4.....	do.....	do.....	do.....	Do.
5.....	do.....	do.....	do.....	Aug. 6
6.....	do.....	do.....	do.....	Aug. 8
7.....	Aug. 7	Aug. 9	Aug. 15	Aug. 19
8.....	do.....	Aug. 10	do.....	Do.

TABLE XI.—Duration (in days) of instars of Colorado potato-beetle larvæ

Experiment No.	First instar.	Second instar.	Third instar.
1.....	3	4	3
2.....	3	3	2
3.....	3	2	4
4.....	3	2	4
5.....	3	2	2
6.....	3	2	4
7.....	2	6	4
8.....	3	5	4
Maximum duration.....			2
Minimum duration.....			6

## FALL MATING FOR SPRING EGG LAYING

The fact that the Colorado potato beetle may be observed mating in September in the latitude of the District of Columbia has probably given rise to the opinion that a third generation might be produced elsewhere—e. g., in Minnesota. This last generation, whether second or third, has been proved in one instance to be fertilized in the fall, the females on issuing being capable of depositing eggs in the spring without a second copulation. This was found to be the case with the generation which held over from 1914 and was observed in the spring of 1915, for a female came to the surface on March 8 and, without mating, deposited eggs on March 11 and 12, which hatched on March 20 and 21. These larvæ fed until March 30 and 31, when they pupated, the adults emerging on April 19,

1915. This was an indoor experiment, and the beetles had been kept in a warm room during this entire period. In the field the first adults were observed in the insectary garden May 4, 1915. It was quite cold during that period compared with the earlier season of 1914.

#### SUMMARY AND CONCLUSIONS

In the authors' experiments in 1914 in the District of Columbia eggs of the Colorado potato beetle were laid almost immediately after the first overwintering beetles were collected in copulation in the spring. These overwintering beetles fed continuously until September 7, when the last one died. The adults of the first generation upon emergence fed for a short time; some of them went into hibernation, but most of them laid eggs for a second generation. Likewise, some adults of the second generation hibernated, while others laid eggs from which adults of the third generation developed. Dr. Chittenden has stated<sup>1</sup> that in the course of his investigations he was not able to get the beetle to breed more than twice in a season without a period of estivation; but from the few eggs that were laid in the second generation the authors were able to rear the species through three generations without a resting period.

In 1908 Popenoe<sup>2</sup> made experiments with this insect in tidewater Virginia, and reared it through three generations, but all the beetles of the third generation died. In this experiment the heat was still greater than in Washington in 1914, and the insects were not isolated in large numbers and were not well fed, which accounts for the dying of the third generation.

The entire developmental period from egg to adult was passed, as previously stated by Dr. Chittenden, in approximately four weeks.

Particular attention is called to the fact that the female, far from laying the small number of eggs attributed to this species, is capable of laying, in one case under actual observation, 1,879, while a second female deposited 1,301 eggs. The former record exceeds any hitherto published, so far as known. It should be stated, however, that during 1913 Mr. W. O. Ellis,<sup>3</sup> of the Iowa Agricultural Experiment Station, obtained from a single female of the species a total of 1,686 eggs, and that Messrs. Girault and Zetek<sup>4</sup> took 1,578 eggs from a single beetle.

From the experiments reported herein it is evident that there are three completed generations of the Colorado potato beetle in the District

<sup>1</sup> Chittenden, F. H. The Colorado potato beetle (*Leptinotarsa decemlineata* Say). U. S. Dept. Agr. Bur. Ent. Circ. 87, p. 8-9. 1907.

<sup>2</sup> Popenoe, C. H. The Colorado potato beetle in Virginia in 1908. U. S. Dept. Agr. Bur. Ent. Bul. 82, pt. 1, 8 p., 2 pl. 1909.

<sup>3</sup> Ellis, W. O. *Leptinotarsa decemlineata* Say. In Jour. Econ. Ent., v. 8, no. 6, p. 520-521. 1915.

<sup>4</sup> Girault, A. A., and Zetek, James. Further biological notes on the Colorado potato beetle, *Leptinotarsa 10-lineata* (Say), including observations on the number of generations and length of the period of oviposition. II, Illinois. In Ann. Ent. Soc. Amer., v. 4, no. 1, p. 74. 1911.

of Columbia and localities having the same mean temperatures, part of the adults of the first and second generations hibernating, while the remainder lay eggs from which the second and third generations develop. Furthermore, the possibility of a partial fourth generation is suggested by the fact that the beetles of the third generation were active and feeding voraciously during September, 1914. This insect is to be found in all stages during the summer months, and there is much overlapping of generations.

22533°—16——2

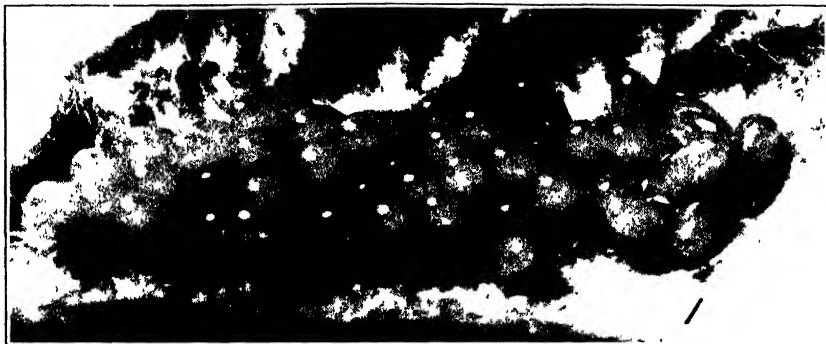
PLATE LXIII

Colorado potato beetle (*Leptinotarsa decemlineata*):

Fig. 1.—Egg mass, highly magnified. Original.

Fig. 2.—Young larva, highly magnified. Original.

(926)





# SOME FACTORS INFLUENCING THE LONGEVITY OF SOIL MICRO-ORGANISMS SUBJECTED TO DESICCATION, WITH SPECIAL REFERENCE TO SOIL SOLUTION

WARD GILTNER, *Bacteriologist*, and H. VIRGINIA LANGWORTHY, *Graduate Assistant in Bacteriology, Michigan Agricultural Experiment Station*<sup>1</sup>

## INTRODUCTION

The following outline is suggestive of the complexity of the problem of determining the relative influence of the various factors affecting the longevity of microbes subjected to desiccation:

- (1) Properties of the organism which probably depend on species differences.
  - (a) Spore formation.
  - (b) Capsule formation.
  - (c) Peculiarities of cell composition.
- (2) Physiological differences in organisms resulting from treatment before drying.
  - (a) Temperature of cultivation.
  - (b) Nutrition.
  - (c) Age of culture.
  - (d) Virulence and other properties.
- (3) Nature of the medium in which the organism is suspended before drying.
  - (a) Its possible plasmolyzing effect.
  - (b) Its content of protective or water-retaining substance.
- (4) Physical structure of the substratum upon which drying occurs.
  - (a) Smooth, nonabsorbent surfaces.
  - (b) Textile fibers or fabrics.
  - (c) Soil, etc.
- (5) Effect of physical agencies.
  - (a) Light.
  - (b) Temperature.
  - (c) Variations in humidity, etc.

Only a few of the points in this outline will be treated in detail in this paper.

## HISTORICAL REVIEW

A review of the literature reveals only the facts that are usually incorporated in recent text books on microbiology. The longevity of spores is too well known to demand discussion at this time. It is recognized in the literature that the presence of a gelatinous capsule is an excellent means of protection against adverse circumstances, especially desiccation. It is also noted by Liesenberg and Zopf (13)<sup>2</sup> that with an organism

<sup>1</sup> This paper represents part of a piece of work planned and prepared for publication by the senior author, but executed almost entirely by the junior author as a part of the requirements for the degree of Master of Science.

<sup>2</sup> Reference is made by number to "Literature-cited," p. 941-942.



like *Streptococcus mesenteroides* the naked modification—i. e., the form developed on a medium containing no sugar and having no capsule—succumbs more quickly as a result of desiccation than does the encapsulated form. *S. mesenteroides* (13, p. 244) has been found to resist desiccation for a much longer period if developed on a saccharin medium than on one which contains no sugar. Revis (20) shows that two types of colon organisms which developed a mucilaginous type of growth were the ones which survived longest in soil. In another article (21) he suggests that the slime formed by organisms of the colon type may add to the water-absorbing and water-retaining capacity of the soil, and may therefore promote the longevity of that organism. Löhnis (15) says that not only the spores but also the bacteria with slimy walls endure the effects of desiccation very well. Lafar (13) emphasizes the importance of making a distinction between organisms like *S. mesenteroides*, which surrounds itself with a gelatinous envelope, and organisms which carry on a slimy fermentation—i. e., conversion of sugar outside the cell into mucinous matter—without themselves being inclosed in capsules. Jensen (11, p. 323) uses the terms capsule formation and slimy fermentation interchangeably and regards the process as protecting the organism against desiccation.

Buchanan (2, p. 378) offers a very comprehensive review of the literature on the nature and morphological origin of bacterial slimes. Some describe gum formation as the result of a true fermentation of carbohydrates by bacteria, calling it an extracellular synthesis, others calling it a true synthetic process, but not necessarily due to an extracellular ferment. Most of the bacterial gums reported in the literature are described as carbohydrates of the formula  $(C_6H_{10}O_5)_n$ . Bacterial slimes classed as dextrans are described by Bräutigam, Kramer, Ritsert, Scheibler, and many others (2). Lipman, Greig-Smith, Maassen, and Laxa (2) found levulan to be the specific gum of several slime-forming bacteria. Schmidt-Mühlheim, Hueppe, Emmerling, Greig-Smith, Laurent, Ward, and Seiler (2) describe bacterial gums having the characteristics of galactans. A few nitrogenous bacterial gums are mentioned, but they appear to be less common than those of a carbohydrate nature. The protective action of these gums has been ascribed to their water-retaining capacity.

Exclusive of organisms with such special protective structures as spores or capsules, it appears to be true that certain species are more resistant than others. Neisser (4) found that the organisms of typhoid fever and diphtheria were the most resistant; cholera, influenza, bubonic plague, and gonococci the least; and the pus-forming cocci, meningococcus, and tubercle bacillus of intermediate resistance. Briscoe (1) credits the tubercle bacillus with a greater resistance than most non-spore-bearing organisms. This power of resistance is no doubt due in part to the waxy or fatty substance found largely in the outer layer of the tubercle bacillus.

Ficker (8) states that the temperature at which the organisms are cultivated and their ability to resist drying at different temperatures stand in a certain relation. Drying at a higher temperature does not always produce a more rapid effect and the drying at a lower temperature a more gradual effect. He concluded that cultivation at a temperature below the optimum produces an individual with the greatest resistance to desiccation. His results (7) with the drying of cholera vibrio cultures of different ages indicate that cultures 1 or 2 days old endure desiccation better than older cultures, but of these two the 48-hour culture is less sensitive to drying at 37° C. than is the 24-hour culture. The results of Kitasato and Berckholtz, quoted in the same article, show about the same resistance in cultures from 1 to 5 days old. Cultures older than these showed a marked decrease in resistance, due not only to the fact that there were fewer living organisms present in the same mass of an old culture, but these surviving organisms possessed in themselves less vitality than did the vibrios from younger cultures. Ficker (7) also demonstrated in the case of the cholera vibrio that a virulent strain was more resistant than an avirulent strain. Ficker's experiments (8) showed that transfers of old cholera vibrios from the surface of agar to distilled water resulted in a disturbance of the turgor of the cell which was so injurious as to make its death, when desiccated, occur much sooner than was the case when they were suspended in physiological salt solution and dried. With young cultures the reverse was true. Suspension in tap water or distilled water appeared to have the same effect, but desiccation after suspension in physiological salt solution was quickly injurious. He explains this on the basis that since the drying process resulted in an increase of concentration of the salt solution, the cell was subjected to both plasmolysis and desiccation. The explanation is not complete, however, for a broth of the same salt content as the physiological salt solution was favorable to both young and old cultures. He found (8) the cholera vibrio to retain its vitality longer when dried from a suspension in milk or broth than in distilled water, tap water, physiological salt solution, serum, or saliva. Ficker (8) also showed that a greater longevity resulted after drying on cover-glass films when the organisms were first cultivated on a solid medium and then suspended in fresh broth or milk, than when they were grown in those liquids and then dried on cover-glass films prepared directly from the medium in which they developed.

Peiser (17) showed that the thermal death point of lactic-acid bacteria when determined in milk is higher than when determined in bouillon. Numerous examples are cited of the long preservation of organisms in a dry state when surrounded by nitrogenous or albuminous material. Chester (4) says that *Pseudomonas radiculicola*, when dried in thin films on glass, perishes very rapidly, but that it may live 11 to 16 days on cotton. Harding and Prucha (23) have shown that *Bacterium campestris* may

live for as long as 13 months on cabbage seeds, but when dried on cover slips it is dead at the end of 10 days. Briscoe (1) says that this difference is no doubt largely due to the difference in the hygroscopic moisture retained by these substances. He found that tubercle bacilli lived only 8 to 12 days when dried in thin smears on glazed-paper slips. *Bacillus coli*, *B. violaceus*, and *B. prodigiosus*, according to his experiments, were even more sensitive dried under those conditions.

As to the relative merits of desiccation in room air and in a desiccator, some fairly positive statements have been obtained. Chapin (3, p. 195) says that as a rule bacteria live longer when dried in a desiccator than when dried in the open air under natural conditions. Ficker (7) showed that the rapid drying of organisms in a desiccator over calcium chlorid or sulphuric acid was preferable to drying in ordinary room air. Ficker's experiment (7), in which the organisms were placed alternately in a desiccator and a moist chamber for a couple of hours at a time, resulted in the organisms so treated dying much more rapidly than did those which were left in the desiccator continuously for the same length of time. Löhnis (15) states that frequent changes between drying and remoistening are most injurious, but that rapid drying in a space with a "rarefied atmosphere" (in a desiccator) is comparatively favorable. Unpublished experiments of J. Simon have shown that the repeated drying and moistening of the soil is much more detrimental to nodule bacteria than keeping the soil constantly dry. Chester (4), in his experiments with *P. radiculicola*, found that an important condition for the successful preservation of the organism in a dry state was to keep the culture sealed from the air and in a dark, cool place.

The evidence obtainable from the literature in regard to the length of time an organism may live in air-dry soil and the factors responsible for its longevity are neither definite nor complete. Lipman (14, pp. 228 and 230) says that—

Under air-dry conditions each soil grain is surrounded by a very thin film of moisture designated as hygroscopic water . . . According to Hall the film of hygroscopic moisture is about  $0.75\mu$  ( $0.00003$  in.) thick . . . Nevertheless, it will be seen that the moisture, even in air-dry material, is deep enough to allow the bacteria a reasonable amount of protection. This will account for the survival of non-spore-bearing bacteria in dry soil for a long time. Indeed, instances are on record of the isolation of *Asotobacter* and *Nitrosomonas* from soils that had been kept in the laboratory for several years.

Löhnis (15, p. 67) says that—

vegetative cells can better endure drying when they are in soil. With spores also this is true. The resistance of spores dried in earth is usually found to be higher than that of spores dried on cotton, silk, glass, etc.

Duggar and Prucha (6) found that after the rapid drying out of soil cultures there remained a large number of living organisms whose vitality

would extend over a considerable period. Nestler (16) investigated an old herbarium and found that even after 23 years 90,000 colonies could be obtained from 1 gram of soil. *Azotobacter* (12) remain alive in soil samples if these samples are kept for 160 days in a desiccator and then 148 days in an air-tight condition. Germano's (9) results seemed to indicate that the organisms of typhoid fever and diphtheria did not live as long in soil as on fabrics, although the diphtheria bacillus averaged 20 to 40 days' longevity in all trials in soil. Firth and Horrocks (3) found that the typhoid bacillus would live for 23 days in dry sand. Pfuhl (18) found the typhoid bacillus to live 28 days in dry sand and 88 days in moist garden earth. The bacillus of dysentery, on which he experimented at the same time, lived only 12 days in sand and 101 days in moist garden earth. Briscoe (1) found the tubercle bacillus to live 213 days in garden soil.

But little work has been done to determine the effect of different soil types on the longevity of organisms dried in them. The data offered in the literature on this point are not only scanty but far from recent. Modern texts hold that dust does not offer protection to many pathogenic organisms, the dangers due to ordinary dust being much exaggerated according to Rosenau (22, p. 72) and Chapin (3, p. 263). Dempster (5) found that the cholera vibrio lived only a short time in perfectly dry soil, but survived for a prolonged period in soil containing a small amount of moisture. The typhoid bacillus showed a greater tenacity of life in soil than did the cholera vibrio, but entire desiccation proved to be quickly fatal to it also. Comparison of the longevity of these organisms in white sand, gray sand, garden mold, and peat showed that with the exception of peat, which apparently contained substances toxic to the organisms, the nature of the soil did not have a direct influence on them. The vitality of the organisms appeared to depend rather on the moisture content of the soil than on its composition. Our experiments on the longevity of soil organisms in different types of soil have led to a modified conclusion. The longevity of vegetative cells in air-dry soil is probably, as Lipman (14, p. 228) suggests, due mainly to the presence of moisture in the hygroscopic form, although undoubtedly the presence of organic colloidal substances with a tendency to retain moisture and with other properties is of importance. Van Suchtelen, in speaking of the analysis of soil solution as quoted by Giltner (10, p. 154), makes certain statements, which, on account of their immediate bearing on this subject, deserve direct quotation. He says:

In many cases there was found in the soil solution a slime. This must be regarded as the first experimental proof of the presence of this substance in the soil, and it is not impossible that much of the irregular behavior of the life in soil can be explained to some extent with a knowledge of this slime. If I may be permitted, I should like to call your attention to the possibility of this substance having an effect on desiccation, diffusion, and other processes.

It is the above statement which has stimulated and formed the basis of the experimental work recorded herein. No progress has been made in the direction of an explanation of the nature of this slime. Its effect on the prolongation of the life of micro-organisms subjected to desiccation has been the object in view.

#### EXPERIMENTAL STUDY

An experiment was conducted to determine whether an organism may receive protection from the solution in which it is suspended before being subjected to desiccation in sand. For this work were used cultures of *P. radicola* grown for five days at room temperature on nitrogen-free ash agar. For suspension the following solutions were employed:

- (1) Physiological salt solution.
- (2) Physiological salt solution + 0.1 per cent of agar.
- (3) Physiological salt solution + 0.1 per cent of gelatin.
- (4) Physiological salt solution + 0.1 per cent of albumin.
- (5) Physiological salt solution + 0.1 per cent of gum arabic.
- (6) Physiological salt solution + 0.1 per cent of soluble starch.

With the exception of the albumin solution these were all prepared by dissolving 1 gm. of the dry substance in a small amount of salt solution and then making it up to a volume of 1,000 c. c. They were found to be practically neutral to phenolphthalein. On account of the difficulty of dissolving powdered egg albumin it was found necessary to use raw white of egg, a quantity being taken which by computation contained 1 gm. of albumin. As albuminous solutions may be heated to 100° without coagulation if slightly alkaline, this solution before sterilization was made -10° F. S. by the addition of N/1 sodium hydroxid. After sterilization (which with all six was accomplished by the Tyndall method, 30 minutes heating in flowing steam on four successive days) the N/1 sodium hydroxid was neutralized with N/2 hydrochloric acid, leaving the albumin solution like the other five, practically neutral.

Suspension of the bacterial growth from four agar slopes was made in 250 c. c. of each of the above solutions. For the purpose of securing initial counts 1 c. c. of each suspension was diluted and plated on nitrogen-free ash agar. Twelve flasks of quartz sand were then inoculated from each of the six solutions, 5 c. c. to a flask. The sand had been prepared after the method described by Rahn (19). It was heated with diluted hydrochloric acid, washed several times, first with tap water and then with distilled water, heated on a water bath until almost air dry, and then heated at least 30 minutes over a free flame. Fifty gm. of the dry sand was placed in 100 c. c. Erlenmeyer flasks, which were plugged with cotton. Sterilization was accomplished by heating for 45 minutes in the autoclave under 15 pounds' pressure.

The inoculated flasks were kept in a dark, well-ventilated place at a temperature of 22° to 25° C. At intervals the number of organisms per

gram of sand was determined by the plate method, samples being taken from two flasks representing each suspension solution. Nitrogen-free ash agar was used for all plates and these were kept 10 days at a temperature of 22° to 25° C. before counting.

It is evident from Table I that the counts are irregular and not such as to form a basis for any positive conclusions. This is due in part to the fact that the fluctuations in numbers from time to time were so extreme that it was difficult to determine what dilutions should be used to obtain plates from which accurate counts might be made. One great mistake in this trial was the addition to the sand of a quantity of moisture which was sufficient to permit the multiplication of the organisms for three weeks after inoculation of the flasks. In later trials the addition of less moisture lessened the period of multiplication. The bacteria were not actually subjected to desiccation until after January 27, by which time the difference in the numbers of organisms developing on the five different substances was such that a fair comparison of their water-retaining capacity during the process of drying was not possible. Although it is true that after a desiccation period extending over almost four weeks (from the last of January to February 24) there were greater numbers of living organisms in the flasks to which the albumin solution had been added, it is possible that this would not have occurred had not the organisms in those flasks reached enormous numbers just previous to the period of drying, because of the superior nutritive qualities of this substance.

TABLE I.—Longevity of *Pseudomonas radicicola*, dried in sand after suspension in different solutions

Date.	Salt solution.	Agar solution.	Gum-arabic solution.	Starch solution.	Gelatin solution.	Albumin solution.
Jan. 2 <sup>a</sup> .....	60,000	60,000	60,000	60,000	60,000	60,000
7 .....	27,400	428,700	30,000	60,500	626,400	—10,000
15 .....	1,711,000	3,651,000	63,160	2,143,000	3,974,000	(?)
27 .....	674,800	328,000	60,000	468,100	1,335,600	3,677,000
Feb. 13 .....	1,000	1,000	—1,000	—1,000	10,000	30,000
24 .....	—50	—50	50	50	—50	200

<sup>a</sup> Initial counts.

Another experiment of the same nature was made with the following solutions:

- (1) Physiological salt solution.
- (2) Physiological salt solution + 0.1 per cent of agar.
- (3) Physiological salt solution + 0.1 per cent of gelatin.
- (4) Physiological salt solution + 0.1 per cent of gum arabic.
- (5) Nutrient broth.
- (6) Milk.
- (7) Soil solution (extracted from garden soil, sandy loam, by the method of Van Soest).

<sup>1</sup> All soil solutions were furnished by Mr. J. Frank Morgan, Research Assistant in Bacteriology.

The bacterial growth from one agar slope was suspended in 12 c. c. of each of the above solutions, and 1 c. c. was diluted and plated quantitatively on nitrogen-free ash agar. From each of the seven suspensions 2 c. c. was added to each of five flasks of quartz sand, which was of the same quality and prepared exactly as in the preceding trial.

These flasks were kept in a dark place at 22° to 25° C. Quantitative determinations, made at intervals, are based on plates from but a single sample of each set, consequently the opportunity for error is materially increased. It can not, therefore, be claimed that these figures (Table II) show accurate comparisons. However, it is quite evident that between March 26 and April 17, during which time the sand was so dry as to make the multiplication of organisms impossible, the rate of decrease in the numbers of organisms taken from broth, milk, and soil solution was noticeably less than that of organisms from the other solutions. This implies a certain protection gained from the presence of nitrogenous or albuminous constituents in the milk or broth. To what substance or substances in the soil solution such protection should be credited can not be stated definitely. The slime, mentioned by Van Suchtelen (10, p. 154), may be of influence in this connection.

TABLE II.—*Longevity of Pseudomonas radicola, dried in sand after suspension in different solutions*

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Broth.	Milk.	Soil solution.
March 18...	1, 100, 000	1, 500, 000	1, 440, 000	1, 613, 000	1, 024, 000	1, 176, 800	1, 460, 000
26...	—10, 000	—10, 000	10, 125, 000	—10, 000	19, 967, 000	185, 000	40, 000
April 6...	—25	25	50	—25	220, 000	405, 000	8, 600
17...	—25	—25	—25	—25	—25	—25	—25

An additional experiment was conducted employing the same cultures used in the previous experiments. The procedure was the same, except that as a basis for quantitative determinations two samples were taken from each set instead of one. As the plates from several of the flasks showed no colonies whatever on May 3, even in the lowest dilutions, which represented 1/25 gm., it was thought advisable in making the next determinations, on May 13, to take 1-gm. samples from these flasks and mix them directly with the melted medium in the Petri dish instead of plating 1 c. c. of a dilute suspension as previously done. It is quite evident that the direct mixture of the sand with the plating medium tends to give higher counts than those secured by plating the washings of the sand, for in the latter case a large number of organisms undoubtedly remain attached to the sand particles instead of being washed off into the suspension. This difference in technic may account for the apparent increase in numbers in certain cases, as shown by the last plating.

TABLE III.—Longevity of *Pseudomonas radiculicola*, dried in sand after suspension in different solutions

Date.	Salt solution.	Agar solution.	Gelatin solution.	Gum-arabic solution.	Albumin solution.	Broth.	Milk.	Soil solution.
April 16.....	1,648,000	2,144,000	1,901,000	3,234,000	360,000	1,477,000	4,026,000	1,266,000
May 3.....	—25	25	—25	—25	56	428,625	515	391
13.....			116		2	432,000	106	3,080

The figures in Table III offer little except a general confirmation of the results of the two other experiments. As the sand was air dry after April 26, it may be understood that the counts on May 3 and May 13 represent the numbers surviving 7 and 17 days desiccation, respectively. Attention must be called to the fact that the lack of figures to show the comparison in increase of bacteria in the different solutions between April 16 and April 26 makes it impossible to overlook entirely the function of these different solutions in their nutritive capacity. Plates were made on April 26, but the nitrogen-free agar made up with maltose instead of saccharose proved an unfortunate choice; for no colonies whatever developed, although, as seen by the two subsequent platings, living organisms were then present in abundance. However, the favorable influence of the soil solution, whether it may be as a food material for soil organisms or a protection during desiccation, can not be disputed.

An experiment was conducted to compare the longevity of *P. radiculicola* dried in quartz sand and in clay-loam garden soil. As in the foregoing experiments, the organism was grown for five days at room temperature on nitrogen-free ash agar. The bacterial growth from one agar slant was transferred to 12 c. c. of physiological salt solution and the mixture shaken thoroughly, and 1 c. c. of the suspension was diluted and plated quantitatively. To the two flasks each of clay loam and quartz sand were added 2 c. c. of the bacterial suspension. The clay loam had been sifted and air dried. The quartz sand had been prepared after Rahn's method, described previously. Fifty-gm. portions of each were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and sterilized by heating in the autoclave for 45 minutes under 15 pounds' pressure.

The inoculated flasks were shaken to distribute the organisms throughout the sand or soil, and then kept in a dark, well-ventilated place at a temperature of 22° to 25° C. The number of living organisms per gram of sand and loam was determined at intervals by plating quantitatively from two samples of each.

TABLE IV.—Difference in longevity of *Pseudomonas radiculicola* dried in quartz sand and in clay-loam soil

Date.	Sand.	Clay loam.
April 16.....	1,648,000	1,648,000
May 3.....	25	42,133
13.....		33,025



It is evident from the data above tabulated that a larger number of organisms survive a limited period of desiccation in clay loam than in quartz sand. This may be partly explained by the difference in grain size and hygroscopic moisture of the two. A given weight of coarse quartz sand consisting of large particles has a surface much less than that of the same quantity of finely divided garden soil, and it therefore retains a much smaller amount of moisture in the hygroscopic form. If the grain size were the only distinction between sand and clay-loam soil, it might properly be concluded that the longevity of organisms in such materials is directly proportional to the percentage of hygroscopic water retained. Such a conclusion is not permissible, however, for the clay-loam soil differs from the sand not only in texture but in content of organic constituents. The amount of such material in any sand is small, and in this case, where the sand was treated with acid, it may be regarded as having been absent. The experiments already described indicate that the soil solution contains substances which offer to the bacteria some protection against desiccation. The soil solution used in our experiments was extracted from just such a soil as was used in the experiment now under discussion.

A further experiment was conducted to compare the changes in numbers and kinds of organisms when soil solution is dried in different types of soils. Soil solution extracted from a rich garden loam was used for this experiment. The soils, obtained from the Soil Physics Department of the Michigan Agricultural College, were of five different types: Muck, sand, sandy loam, clay, and clay loam.

Fifty-gm. portions of these soils in the air-dry condition were placed in 100 c. c. Erlenmeyer flasks plugged with cotton and were then sterilized in the autoclave for 45 minutes under 15 pounds pressure. For greater exactness the total quantity of soil solution was agitated and then divided into five 250 c. c. portions; from each of these 1 c. c. was plated on ordinary agar in dilutions of 1 to 10,000, 1 to 100,000 and 1 to 1,000,000. Ten flasks of each type of soil were then inoculated with the soil solution, all the solution used for any one type of soil being taken from a single flask. Although it was desired to have the inoculum approximately equal in all cases, a quantity of liquid which barely moistened the muck and clay loam was found to more than saturate the coarser soils. So, to make the physical conditions more nearly alike, 15 c. c. of the solution was used for each flask of clay, clay loam, and muck, but only 10 c. c. for the flasks of sand and sandy loam.

The inoculated flasks were kept on a shelf in the laboratory at a temperature of 20° to 25° C., exposed to very dim diffused light, and subject to the influence of normal variations in the humidity of the room atmosphere. At intervals of about four weeks quantitative determinations were made, samples being taken from two flasks of each soil. After the first plating, samples were taken from one flask opened at the previous

plating and from one new flask each time, the object being to secure more representative counts. Plates were made with ordinary agar and kept for one week at a temperature of 22° to 25° C. before counting.

Moisture determinations were made in duplicate at the time of each quantitative plating, the bacterial counts being then computed on the oven-dry basis.<sup>1</sup> Small variations in the percentage of moisture, occurring after the soils attained the air-dry condition (which with sand and sandy loam was by March 3 and with the other three soils between March 3 and March 29), are probably the result of fluctuations in the humidity of the room air. In the case of clay it was impossible to secure a thoroughly mixed sample, owing to its drying into a sort of hard, baked condition; therefore, a slight irregularity in the moisture determinations could not be avoided. The data are recorded in Table V.

TABLE V.—*Number of bacteria per gram in 50 grams of sand, sandy loam, clay, clay loam, and muck when dried after the addition of soil solution*

Date.	10 c. c. of soil solution added.				15 c. c. of soil solution added.					
	Sand.		Sandy loam.		Clay.		Clay loam.		Muck.	
	Number of bacteria per gram.	Per-cent-age of water.	Number of bacteria per gram.	Per-cent-age of water.	Number of bacteria per gram.	Per-cent-age of water.	Number of bacteria per gram.	Per-cent-age of water.	Number of bacteria per gram.	Per-cent-age of water.
1914:										
Nov. 17	285,200	20.0	170,000	20.0	462,900	30.0	225,000	30.0	453,900	30.0
Dec. 29	4,318,000	14.54	26,170,000	14.38	11,500,000	28.96	60,840,000	31.81	33,689,000	26.13
1915:										
Jan. 28	1,912,000	6.25	5,806,000	2.81	1,492,000	19.17	26,006,000	16.96	16,613,000	24.85
Mar. 3	197,000	.1	1,555,000	.84	914,000	3.59	12,798,000	9.83	5,782,000	19.51
29	51,900	.36	1,967,000	.78	552,000	.93	4,659,000	2.93	4,924,000	16.33
Apr. 21	18,900	.16	1,066,000	.84	447,100	1.57	4,135,000	3.31	4,217,000	16.32
May 7	32,500	.27	983,000	1.08	278,800	1.81	3,845,000	3.65	2,220,000	16.25
14	37,000	.22	2,245,000	1.10	378,000	1.74	3,914,000	3.63	2,703,000	15.91
June 18	41,000	.20	3,218,000	1.22	494,000	1.98	5,456,000	4.26	1,836,000	16.80
Sept 6 <sup>a</sup>	127,600	.14	6,523,000	1.23	1,241,000	2.26	11,686,000	4.30	2,781,000	16.78

<sup>a</sup> This count was made by Mr. O. M. Gruzit, Graduate Assistant in Bacteriology.

With a view to determining the predominant types of organisms placed in the soils, isolations were made from a few of the most common types of colonies occurring on the plates of the original soil solution. The characteristics of these organisms were studied. It must not be assumed, however, from the fact that so few organisms were isolated, that the flora of the soil solution was limited to the species observed. The high dilutions necessary for obtaining accurate quantitative plates failed, of course, to show up the organisms which were present in smaller numbers. From the quantitative plates made after the soils reached the air-dry state, between March 3 and May 7, isolations were made of the most numerous types. As the muck plates were frequently overgrown with a downy white mold, but few pure cultures could be obtained from

<sup>1</sup> Dried at 105° C. for 24 hours.

that source. As seen in Table V, the loam soils and muck show a higher count six months from the time of inoculation than do the clay and sand. During the first six weeks all five soils contained an amount of moisture sufficient for bacterial growth, and during the last two months only were the soils in the air-dry state. The amount of activity in the period intermediate between the optimum and minimum supply of moisture shows a gradual decrease, the rate varying in the different soils.

While there was not a great difference in the initial counts, the opportunity for bacterial growth in the five types of soil was by no means the same. This is clearly evidenced by the contrast between their counts during the first period, when the moisture content was yet sufficient to permit multiplication. Since the sand was saturated with the amount of soil solution used as an inoculum, it at first presented conditions more favorable to anaerobic than to aerobic species. As this amount of moisture diminished and the oxygen supply increased, opportunity for the growth of aerobic types was given, but the extent of this favorable period was limited not only by the small amount of organic food material but also by the extremely rapid evaporation of moisture. Conditions in the clay were at first comparable with those in the sand, it being practically waterlogged. With the gradual reduction in moisture and increase in aeration, the growth of aerobic and facultative bacteria proceeded. The smaller size of the grains produced two noticeable effects—viz, a limited oxygen supply, inhibitory to the extensive multiplication of aerobic species, and a prolonged retention of moisture, which favored the longevity, if not the activity, of non-spore-bearing bacteria. As in the sand, a low content of organic nutrients acted as a natural limit to the growth of saprophytic species. In the clay loam, sandy loam, and muck multiplication was possible from the start, for the amount of solution used for inoculation was just sufficient to moisten the soils without saturating them. Their higher content of organic substance also gave them an advantage in respect to nutrition.

However, in these soils also differences in size of grain, thickness of moisture film, and oxygen supply proved to be factors of more influence than the mere abundance of organic food substance. The muck, for instance, although containing the highest percentage of such organic materials, proved to be of a less favorable medium for bacterial growth than did the clay loam. The grain size of the clay loam appeared to be that which was most advantageous with respect to aeration, thickness of moisture film, and retention of hygroscopic water. Its content of decomposable substances, while not so great as that of the muck, was more than sufficient for microbial development. The sandy loam, with a smaller amount of organic materials, somewhat larger grain size, and consequently less hygroscopicity, did not show as large numbers of living

organisms at any time as did the clay loam, although its oxygen supply in consequence of these same conditions must have been somewhat greater.

We therefore perceive that the optimum condition for microbial activity in soil is a proper adjustment of these previously mentioned factors. With regard to longevity, fewer factors are concerned, the data so far obtained indicating that it is a function of both grain size (and therefore amount of hygroscopic moisture) and content of organic substances.

The influence of soil type was made evident not only in the numerical counts but also in the varieties of organisms persisting in the different soils throughout the two months during which they were in the air-dry state. As the condition of the sand had been such as to favor the development of organisms with high oxygen requirements, plates of high dilution always showed a predominance of those types. Such of these as were spore bearers became a larger and larger proportion of the total number, as the period of desiccation extended and the non-spore-bearing species died out. Among the spore bearers most frequently found were *Bacillus mycoides* and aerobes of similar morphological and cultural characters. Of the non-spore-formers an organism found in larger numbers than any other single species showed the greatest longevity. The characteristics of this organism are as follows:

It is a rod with rounded ends,  $0.6\mu$  by  $1.3$  to  $1.5\mu$ . It is actively motile, non-spore-forming and non-capsule-forming. It is frequently observed in pairs. It stains readily with aqueous alcoholic fuchsin. In nutrient broth it produces a decided turbidity, some sediment, and a soft surface scum. The growth on agar is glistening, translucent, grayish white, and very abundant. On a gelatin stab there is a white surface growth, with a filiform growth in the stab, but not liquefaction. Litmus milk becomes bluer after 48 hours; some peptonization in 30 days. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Facultative anaerobe. Optimum temperature,  $25^{\circ}\text{C}$ . Habitat, soil.

Physical conditions in the clay had somewhat inhibited the extensive multiplication of strongly aerobic types, but permitted the development of facultative bacteria. Since anaerobic organisms could not be secured by the method of plating used, no mention of them is possible. As the non-spore-bearing types declined, the plates showed more evidence of spore-bearing, strictly aerobic varieties similar to those met with in the sand. The fact that such colonies were not found until their diminishing numbers necessitated the use of lower dilutions suggests their development from spores which had merely remained latent in the clay without passing through a process of multiplication and subsequent destruction like the majority of the facultative non-spore-bearing species. The non-spore-forming organism showing greatest endurance of desiccation was a type identical with that persisting in the sand.

During the period of extensive multiplication, the plates from sandy loam, clay loam, and muck showed quite similar types, although the sandy loam has slightly greater numbers of the strongly aerobic spore-forming species. As the numbers diminished, spore-bearing types became more frequent on plates from both sandy loam and clay loam, but were not evident on the plates from muck. It is to be inferred that the multiplication of those in the finest soil had not progressed to such an extent as to make their colonies numerous in high dilutions, their numbers apparently being in proportion to the grain size and amount of aeration. The most persistent non-spore-bearing organism was of the type already referred to, as found in clay and sand. In addition to this, certain chromogenic cocci and one variety of slime-forming organism were frequent on plates from all three of these soils through the time of desiccation. This slime former, which was especially numerous on plates from muck, is described as follows:

The organism is a rod  $0.4\mu$  by  $0.6$  to  $0.7\mu$ ; nonmotile. No spores observed. No capsule demonstrated. Stains readily with aqueous alcoholic fuchsin. Nutrient broth made slimy and very turbid. Growth on agar spreading, translucent, orange-yellow, slimy. Gelatin stab, surface growth and rapid liquefaction. Litmus milk discolored, alkaline, slimy; peptonization begun in 48 hours and complete in 10 days. Facultative anaerobe. No indol from Dunham's peptone solution. Ammonia produced from Dunham's solution and nitrates reduced. Habitat, soil.

Attention should be called to the rather peculiar circumstance that not one of the organisms isolated during the last two months corresponds to any one of the four organisms which predominated in the original soil solution used for the inoculation of the five soils. The extinction of these species may have been due either to the unfavorable influence of association with other organisms during the period of active multiplication or to their lack of endurance when supplied with less than the optimum amount of moisture.

#### CONCLUSIONS

(1) The survival of non-spore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain size and hygroscopic moisture.

(2) Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand, under the conditions of our experiment.

(3) If bacteria are suspended in the solution extracted from a rich clay loam before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in physiological salt solution.

(4) The solution extracted from a rich clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

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# OBSERVATIONS ON THE LIFE HISTORY OF THE CHERRY LEAF BEETLE

By GLENN W. HERRICK, *Entomologist*, and ROBERT MATHESON, *Assistant Entomologist*, Cornell University Agricultural Experiment Station

## INTRODUCTION

The cherry leaf beetle (*Galerucella cavicollis* Lec.), which attracted much attention during the season of 1915, is a native insect that has adopted several new food plants, at least in the beetle stage. Not since the first record of its work on cultivated plants, in 1894, has its injury been as great or as widespread as during the summer just past. It would seem that the early prediction of Davis (2),<sup>1</sup> who first recorded the beetle's work on cherry (*Prunus* spp.), was about to be fulfilled, that as it was a northern and widespread species we might expect it to become increasingly injurious from year to year.

## HISTORICAL REVIEW

The cherry leaf beetle was originally described by Le Conte in 1865 (5, p. 216) from a single specimen received from North Carolina. Nothing further is recorded of this beetle till 1890, when Packard, who found this species in large numbers at Berlin Falls, N. H., eating holes in the leaves of wild cherry, probably the pin cherry (*Prunus pennsylvanica*), refers (7, p. 529) to it under the name "*Galeruca sanguinea*."

The next reference is by Davis (2), who reports it as being abundant at Bellaire, Mich., during the summer of 1894 and destroying the foliage of cultivated cherries. This is the first record of this beetle's attacking the foliage of cultivated trees, and Davis makes the suggestion that as this insect is a northern species it may yet become quite injurious. The larvæ were found in this same locality; but it is not stated on what plants they were feeding, though the writer states that wild cherries were only a short distance away.

Lintner (6) records this beetle as occurring in thousands on June 10, 1895, at Ausable Forks, N. Y., feeding on the foliage of the cherry left uninjured by late frosts. He also states that his correspondent found this same insect at work early in July on the foliage of young chestnut trees, but that he did not verify this observation.

Felt (3), in 1898, records outbreaks of this insect at Corning, N. Y., the beetles occurring in such numbers as to threaten the destruction of the trees. Smith was the first to record the occurrence of this beetle on peach, having found it in Pennsylvania during the summer of 1898.

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<sup>1</sup> Reference is made by number to "Literature cited," p. 949.



Johnson (4) reports an extensive outbreak on "fire cherry" (*Prunus pennsylvanica*) at Ricketts, Wyoming County, Pa., during September, 1897, the beetles and larvæ occurring in immense numbers.

Chittenden (1) reports outbreaks of this beetle in June, 1898, at St. Ignace, Mich., on cherry and at Spruce Creek, Huntington County, Mich., on young peach trees. He states that larvæ are known to feed on cherry and probably also on peach, but mentions no definite records of such occurrences on the peach.

Since the publication of Chittenden's article, nothing has been recorded of this insect, and undoubtedly during all the years since 1898 no injury of any consequence has been committed by it.

#### OUTBREAKS IN NEW YORK IN 1915

During the summer of 1915 several severe outbreaks occurred in New York, the beetles defoliating cherry, peach (*Amygdalus persica*), and plum (*Prunus* spp.). On June 3 Mr. E. P. Putnam, of Jamestown, N. Y., wrote the Entomological Department, inclosing specimens of beetles, saying that they were defoliating wild cherry and peach trees in the park and also reported them as seriously defoliating cherry and peach trees throughout the town and neighboring districts. On June 11, Mr. H. B. Rogers reported them as injuring cherry and peach and later wrote that this beetle had done considerable injury throughout Chautauqua County. Reports of injury have been received from the following localities: Sonyea (cherry, peach, and plum); Perry, Scio (cherry); Olean, Honeoye Falls (cherry); Bath (cherry); Holland (cherry); Collins, Gowanda, Wyoming, Batavia (cherry and peach); Perrysburg (cherry); Jamestown (cherry and peach); Chautauqua (cherry and peach); Kennedy, Fredonia, Ripley (plum and peach); Castile (cherry); Elmira (cherry and peach); Hornell (cherry); and Ithaca (cherry and peach). All these reports came during the month of June and early in July and nothing has been heard of later injury. Evidently the beetles have not bred locally in such numbers that the work of the adults would attract attention in August and September.

The causes which brought about so widespread an outbreak of this insect can not at present be determined. Practically all the injury was restricted to western and southwestern New York. It has been suggested that the beetles migrated northward from Pennsylvania, but this does not seem plausible, as the native host, *Prunus pennsylvanica*, is a northern tree, occurring southward only as far as Pennsylvania and in the mountains to North Carolina and Tennessee. Conditions must have been favorable for the increase of this beetle in 1914 and hibernation must have been attended with little loss of life, resulting in such large numbers of the overwintering beetles as to cause overcrowding of the normal food plants. Should favorable conditions prevail during any year, we may again look for a sudden and perhaps more widespread outbreak.

## LIFE HISTORY AND HABITS

The cherry leaf beetle is a pretty, dull-red beetle measuring 4.5 to 5.5 mm. in length (Pl. LXIV, fig. 1). The antennæ are black, and the legs vary from almost black to nearly reddish in color. There are no strikingly distinguishing characters, but the coloring will nearly always serve to separate it from the more closely related northern species. The beetle is widely distributed, occurring from Canada through the New England States southward into Pennsylvania and west to Wisconsin. Chittenden (1) also records it from Texas and Vancouver, British Columbia. The original specimen described by Le Conte (5, p. 216) is from North Carolina.

This insect is one of our native beetles and up to 1894 had only been recorded on wild cherry. In that year it was found attacking the cultivated cherry, destroying the foliage. Later Smith (8) recorded it as injuring peach, and this year it has been reported as feeding on plum. How much more extended the feeding habits of this beetle may become can not even be guessed, though its future destructiveness will depend largely upon whether the larvæ can also adapt themselves to new and closely related food plants.

The beetles pass the winter in hibernation and, although the time of emergence has not been determined, they probably appear in May or, if the weather is favorable, during the latter part of April. They feed actively during May and June not only on the pin cherry but also on the peach, cherry, and in some instances the plum (Pl. LXV, fig. 5). In the field the beetles began to leave the cultivated food plants early in July and practically all had gone by the middle of the month.

In New York State there is only a single brood a season. The new brood of adults appears during the second week in August and becomes common during the latter part of the month and early September; they feed almost exclusively on the pin cherry and do not seem to migrate far from their host plant. In our rearing cages they began entering the soil or crawling under stones about the middle of September, but on fine days would return to feed on the pin-cherry foliage. In early October they had all entered hibernating quarters and did not leave them even on the finest or warmest days.

The work of the beetles is most noticeable during June and early July. After the middle of July the beetles had largely disappeared from the cultivated trees about Ithaca. Although many adults had been seen in copula, no eggs were observed, despite a close watch on all their new food plants. It was supposed that in accordance with the habits of closely allied species, as the elm leaf beetle (*Galer cella luteola*), the eggs would be found on the host plant.

On July 21 Mr. Cotton, a student in the Entomological Department, found adults and what he considered larvæ of this species on pin cherry.

On examination it was at once seen that there were larvæ in all stages, but the closest search did not reveal a single egg on the foliage or trunk or branches of the tree. The youngest larvæ, which seemed to us to have just hatched, were very active, running about over the trunk and branches, and a search at the base of the trees soon revealed immense numbers of eggs just below the surface of the soil, in the matted grass, under sticks, and among rubbish.

#### THE EGG

We did not observe the date of the first egg laying nor determine the number of eggs laid by a single female. At Ithaca egg laying occurs from June to August. If we judge from the length of the larval life and the egg stage, the deposition of eggs at Ithaca undoubtedly began the last week in June. The egg-laying period extended throughout July and the early part of August.

The egg is entirely different in shape from that of closely allied species. It is oval, of a light-straw color, and measures 0.72 to 0.84 mm. in length by 0.60 to 0.64 mm. in width. The entire surface is marked with rather regular hexagonal areas. Large numbers of these eggs were found at the base of the few pin-cherry trees located close to the Cornell University grounds. The eggs adhered rather firmly to each other and to the matted grass. Although close search was made, no eggs could be found at the base of any other species of *Prunus* (Pl. LXV, fig. 1, 2).

#### THE LARVA

During the latter part of July eggs hatched in from 14 to 18 days after they were laid. The young larva escapes from the egg by cutting a hole through one side with the mandibles. The young larvæ are very active, running about rapidly. They soon find their way to the trunk of the tree and could be seen any time during the hatching period clambering actively over the branches in search of the young and tender foliage near the tips of the twigs. They are found most commonly on the under surface of the foliage skeletonizing the leaves. They feed ravenously, grow rapidly, and reach maturity in from two to three weeks. Where the larvæ are abundant all the foliage may be so completely skeletonized as to turn brown and die, giving the trees a scorched appearance (Pl. LXV, fig. 3, 4). The length of the life cycle, with the number of molts, is given in Table I.

TABLE I.—Length of life cycle and number of molts of the cherry leaf beetle

Eggs.		First stage.	Second stage. *	Third stage.	Entered soil to pupate.	Emergence of adult.
Laid. <sup>1</sup>	Hatched.					
.....	July 23	July 30	Aug. 3	Aug. 9	Aug. 10	Aug. 28
.....	do.....	July 29	do.....	do.....	do.....	Do.
.....	do.....	July 30	Aug. 4	Aug. 8	Aug. 9	Aug. 26
.....	do.....	July 28	Aug. 1	Aug. 4	Aug. 5	Aug. 24
.....	do.....	July 27	do.....	Aug. 6	Aug. 7	Aug. 25
.....	do.....	July 29	Aug. 3	Aug. 8	Aug. 9	Aug. 27
.....	do.....	do.....	Aug. 1	Aug. 7	Aug. 8	Aug. 26
.....	do.....	July 30	Aug. 2	do.....	do.....	Aug. 27
.....	do.....	July 28	do.....	do.....	do.....	Aug. 28

<sup>1</sup> From another series of experiments the length of the egg stage was determined. The eggs hatched as follows: 15, 18, 17, 18, 16, 18, and 14 days after they were laid. The average is 16 days. If this were taken as the average length of the egg stage, the total length of the life cycle from the egg to the adult would vary from 48 to 53 days.

## DESCRIPTION OF LARVAL STAGE

**FIRST INSTAR.**—The newly-hatched larva is depressed, fuscous in color, the head, thoracic shield, legs, and anal segment, black. Scattered over the larva are a number of setæ. Length, 1.4 to 1.6 mm.; greatest width, 0.45 to 0.50 mm.

**SECOND INSTAR.**—Nearly cylindrical, slightly depressed, fuscous to brown in color, the head, legs, thoracic and anal shields black. The ground color is almost entirely obscured by the black areas as shown in Plate LXIV, figure 2. On each segment, except the prothoracic and anal, there are two oval, rather sharply defined, large, black areas separated from each other by a narrow line. Laterad of the black areas are angular black markings as shown in Plate LXIV, figure 2. Length, 2.5 to 3.5 mm.

**MATURE LARVA, THIRD INSTAR.**—Length, 6 to 8 mm., nearly cylindrical, somewhat depressed, with an average width of about 2 mm. (Pl. LXIV, fig. 3). The larva after the second molt measures 5 mm. in length and is black in color. As it feeds, the black spots and markings become separated and the brownish yellow ground color shows distinctly. Head black, narrower than thorax; mouth parts yellowish brown. Legs, prothoracic and anal shields black. Dorsally each segment, except the prothorax and anal segments, with two sharply defined oval to rectangular black areas separated by a brownish yellow line; laterad of each of these there is an angular black spot and beyond each of these a smaller rounded black mark. Along the lateral margin there is an elongate oval black spot on each segment. The venter of each abdominal segment is marked with five dark brown to black spots, the central one being largest. The prosternum is black; meso- and meta-sterna each with a narrow, elongate, black area in front and two black rounded spots just caudad of it.

## FOOD HABITS OF THE LARVA

From a close examination about Ithaca we failed to find the larvæ present on any trees but the pin cherry. The few trees of this species located near the campus were swarming with the beetles and larvæ. However, on the other food plants of the adult we found, late in the season, only a few beetles and no larvæ. To determine whether the larvæ could survive and reach maturity on the other species of *Prunus* the following experiments were performed:

EXPERIMENT 1.—On July 23 six larvæ, some almost mature, were placed on the leaves of *Prunus avium*. Two died on July 25, two more on the 27th, and the remaining two entered the soil to pupate on July 28, the adults emerging on August 15. The immature larvæ did not feed, but the nearly mature forms fed slightly before entering the soil to pupate.

EXPERIMENT 2.—On July 23 two young larvæ were placed on leaves of *Prunus avium*. Both died on the 26th without having fed at all.

EXPERIMENT 3.—On July 27 three half-grown larvæ were placed on leaves of *Prunus virginiana*. On the 28th all had left and entered the soil in an attempt to pupate. Later all failed to pupate and died.

EXPERIMENT 4.—On July 27 five half-grown larvæ were placed on leaves of *Prunus virginiana*. On July 28 one was dead and the others entered the soil. All failed to reach maturity.

EXPERIMENT 5.—On July 28 three half-grown larvæ were placed on leaves of *Prunus serotina*. All failed to feed and died on July 31. On the same day four more half-grown larvæ were placed on leaves of *P. serotina*. All failed to feed and died on July 30.

It will be seen from the above experiments that the larvæ seem to be unable to survive on either the cultivated sweet cherry (*Prunus avium*) or the common two native varieties *P. serotina* and *P. virginiana*. It is unfortunate that through an oversight experiments were not made with the other species of *Prunus*. The food plants of the larvæ are undoubtedly restricted at the present time to the wild red, or pin, cherry. Whether the larva can succeed in adapting itself to other host plants seems to be a doubtful question, so that in the future the abundance of the beetles will depend not so much on the presence of its enemies as on a goodly supply of the larval food plant.

## THE PUPA

Pupation takes place at or slightly below the surface of the soil. No special preparation is made by the larva, the pupa often lying openly on the surface in the grass or under rubbish. The pupa is bright yellow, strongly convex, without any distinguishing markings. Scattered over it are small, short brownish tipped setæ, which aid in preventing injury from the soil. The tip of the abdomen is furnished with two diverging strong black spines (Pl. LXIV, fig. 4).

## CONTROL OF CHERRY LEAF BEETLE

On account of the comparatively small numbers of the beetles at Ithaca, we were not able to conduct control experiments. However, several of our correspondents have had good success with lead arsenate (paste) used at the rate of 4 to 5 pounds to 100 gallons of water and also with a spray containing 40 per cent nicotine. In the case of the nicotine spray our correspondent used it at the rate of 3 pints to 100 gallons of water and reported good success. He also reports failure with lead arsenate, though using treble and even quadruple the quantities generally recommended for foliage-feeding insects.

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PLATE LXIV

*Galerucella cavicollis*:

- Fig. 1.—Adult.
- Fig. 2.—Larva, second instar.
- Fig. 3.—Larva, third instar.
- Fig. 4.—Pupa.

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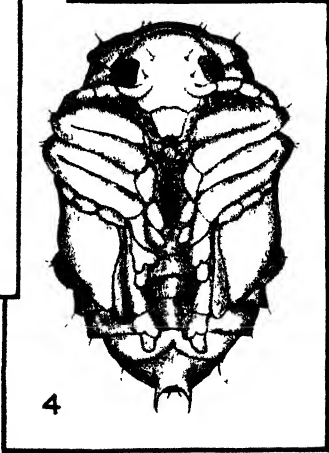
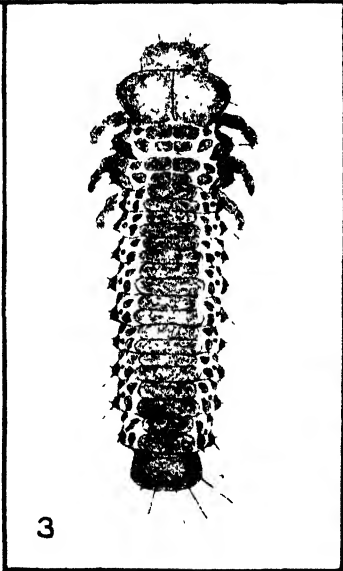
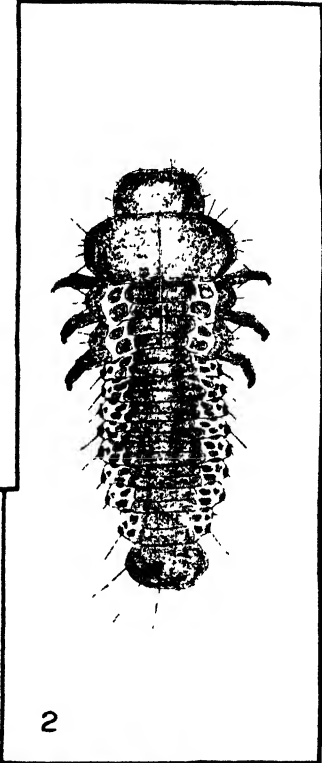
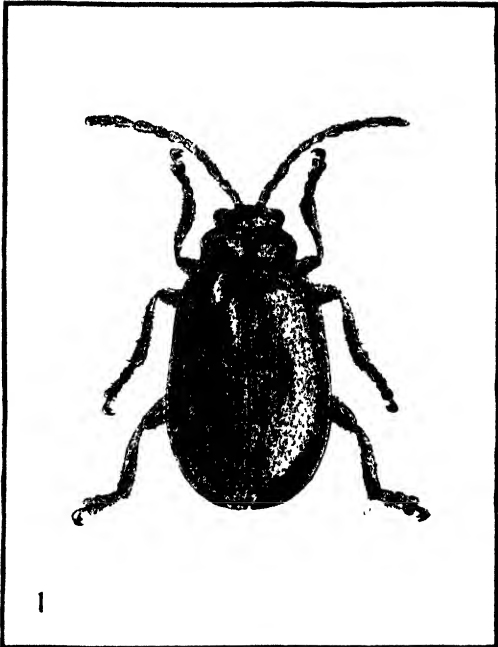






PLATE LXV

*Galerucella cavicollis:*

- Fig. 1.—Eggs on ground at base of tree.
- Fig. 2.—Eggs, enlarged.
- Fig. 3.—Larvæ feeding on leaf.
- Fig. 4.—Work of larvæ on foliage.
- Fig. 5.—Work of beetles on foliage.



# APPARATUS FOR MEASURING THE WEAR OF CONCRETE ROADS

By A. T. GOLDBECK,

*Engineer of Tests, Office of Public Roads and Rural Engineering*

Many miles of concrete roads have been built during the past few years, and the methods employed in their construction are rapidly becoming standardized. The concrete mixture is now made comparatively rich, and in general the aggregates are selected with as much care as present knowledge of these materials permits. Even yet, however, it is doubtful whether the right mixture is being used for the purpose: Whether it is too rich for economy or whether it should be made still richer. It is questionable what kinds of coarse aggregates give the most economical results: Whether they should be composed of hard, tough fragments of trap rock or of softer, more friable pieces of limestone of approximately the same degree of hardness as the mortar in which they are embedded; whether angular fragments of crushed stone should be used or whether round pieces of gravel are equally satisfactory. Definite knowledge on these points based on scientific information seems to be lacking.

The ideal concrete road should wear uniformly and slowly. When due care is exercised in construction and the necessary precautions are taken in maintenance, uniformity of wear may to a large extent be controlled. But little is known about the rate of wear of concrete roads having various aggregates and carrying different kinds of traffic. General observation indicates that some roads with particular kinds of aggregates are wearing more slowly than others containing different coarse aggregates, even though the traffic conditions are nearly alike. We have, however, no definite idea of the amount of wear in these different roads. There must come a time in the life of every concrete road when, notwithstanding careful maintenance through crack protection and patching, its thickness will approach the minimum, making imperative the expenditure of a considerable amount of money for a new wearing surface to replace that gradually worn away by traffic. Every fractional part of an inch decrease in thickness therefore represents a very definite depreciation in the value of the pavement. Money can not be expended intelligently on various aggregates mixed with cement in different proportions for road construction without accurate knowledge of one of the most important factors governing the expenditure—namely, the probable rate of depreciation of the road as determined by actual wear.

This consideration has led the Office of Public Roads and Rural Engineering to attempt to gain definite knowledge of the wear of concrete roads carrying various kinds of traffic, and a special instrument has been designed by the writer and built in this office for that purpose. Several methods of taking autographic records of the cross section of the road were considered, but were discarded in favor of the simpler and more portable form of instrument finally constructed.

Essentially, this instrument consists of a fine wire stretched tightly across the road at a constant height, together with an inside micrometer for measuring the distance from the road surface to the wire. Measurements taken 1 foot apart across the road permit the plotting of its cross section, and if these measurements are repeated at long intervals the change of cross section or the decrease in the thickness of the road will be revealed.

The accompanying illustrations show the instrument in detail and its method of application on the road. If Plate LXVI, figure 1, and text figure 1 are first referred to, the component parts of the apparatus may be seen very plainly.

Pieces *A* and *B* are made of cement mortar and have embedded in them steel rods, *C*, drilled with holes slightly inclined with the horizontal. A fine piano wire about 0.01 of an inch in diameter is passed through these holes and is stretched across the road from block *A* to block *B*. The tops of these rods are each provided with a disk-level bubble, so that when placed in position in the road the rods may be adjusted to a vertical position. Block *A*, which is heavier than block *B*, is provided with two adjusting screws, *D*, for adjusting rod *C* to the vertical. Block *B* rests on two points only, one the lower end of rod *C* and the other the end of adjusting screw *D*. Constant tension is produced in the wire by the weight of block *B*, which is pivoted about the bottom of rod *C* and is adjusted to a horizontal position by means of rack *E*, provided at the end of the wire. As the weight of block *B* is constant, the tension in the wire, and consequently the amount of sag for like spans, must remain the same. A very definite and fixed datum is thus provided, which should remain constant from year to year and which is very easily established by merely placing the end blocks of the apparatus in their proper position on the road.

The bottoms of rods *C* are spherical in shape; and when in use on concrete roads, they rest on the flat tops of bronze plugs cemented in the road surface. These plugs are  $\frac{1}{2}$  inch in diameter and are  $1\frac{3}{4}$  inches long. They are set  $\frac{3}{4}$  inch below the surface, and their tops are protected by means of a brass pipe plugged with a bituminous-sand mixture during the long intervals between readings.

In obtaining the wear measurements a chalk line is first snapped across the road between the bronze plugs, and the points at which it is

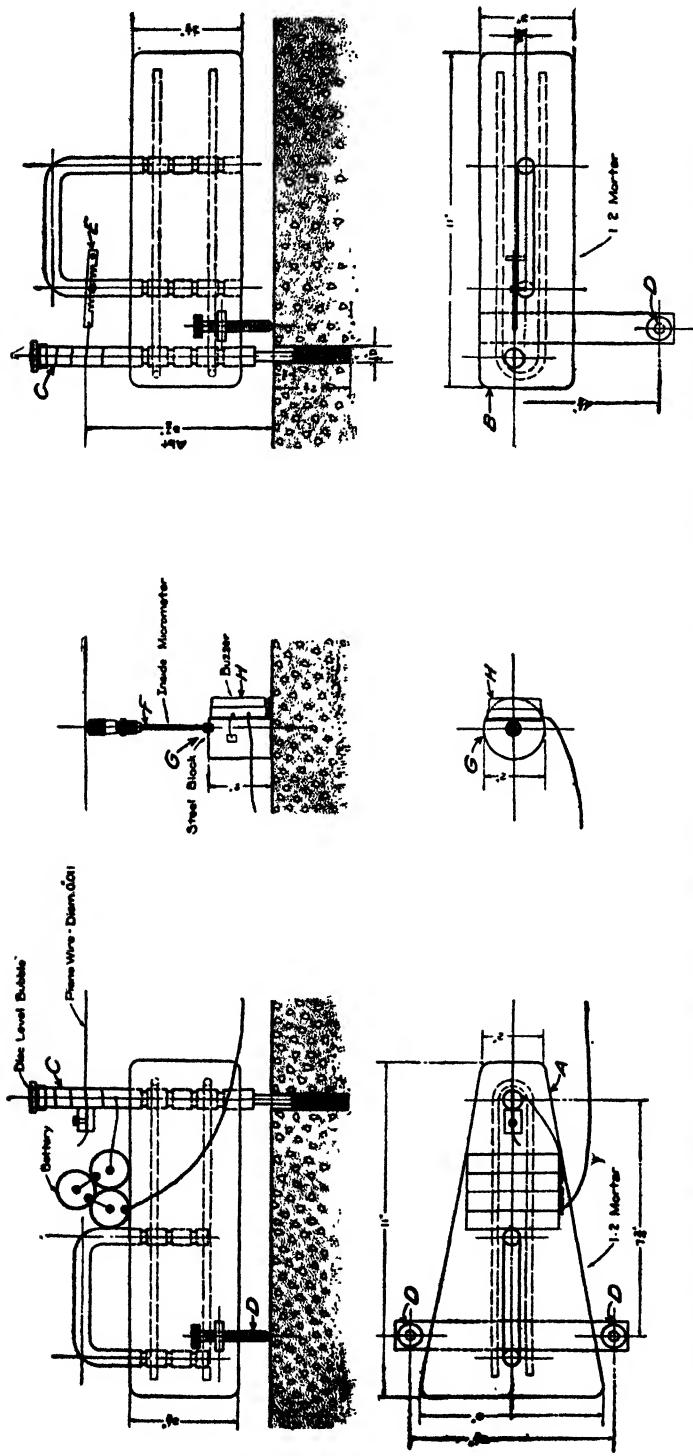


FIG. 1.—Details of instrument for measuring the wear of roads: A, Heavy mortar block; B, light mortar block; C, steel rod; D, level adjusting screws; E, adjusting rack; F, inside micrometer; G, steel bearing block; H, electric buzzer.

purposed to take readings are marked on this line. At these points a steel block, *G*, 2 inches in diameter, is placed, in order to avoid measuring the small local inequalities in the road surface. In the top of this block a flat-bottomed cylindrical recess is made, and an ordinary inside micrometer is held in the recess, while its upper end is adjusted to contact with the steel wire stretched across the road. An electric buzzer, *H*, is mounted on the side of this block, and when contact is made between the micrometer and the wire an electric circuit is completed through the buzzer. With this instrument readings for wear may be taken to the nearest 0.001 inch, although this degree of accuracy will not be necessary.

Holes in the road in which the bronze plugs are set are drilled by means of a special hand-operated drill press carrying a star drill.

In Plate LXVI, figure 2, the method of mounting the apparatus in the road and its manipulation are plainly shown. On the left is the heavier end block carrying the batteries, and on the right is the lighter block the weight of which supplies constant tension to the fine steel wire, part of which is seen in front of the operator. The cord extending on the road surface from the heavier block to the small steel block carrying the micrometer is one of the leads from the battery to the electric buzzer.

Placing the buzzer in this position near the operator obviously is advantageous, especially when the instrument is to be used amidst the distracting noises of traffic. The end blocks are set as near to the sides of the road as practicable, in order to permit measurements being taken across almost the entire width of the road. Should longitudinal cracks develop through the sections measured, the readings so taken will be rendered useless; and in order to eliminate this difficulty, sufficient plugs must be set to permit obtaining readings at uncracked sections.

Wear measurements of this kind taken of the actual road surface should prove of great future value if the traffic conditions and the physical characteristics of the concrete materials likewise are known, and should help to decide present moot questions regarding concrete roads and road materials. Not only may concrete surfaces be measured for wear in this manner, but the wear or vertical movement of other kinds of road surfaces may likewise be determined by the use of this instrument.





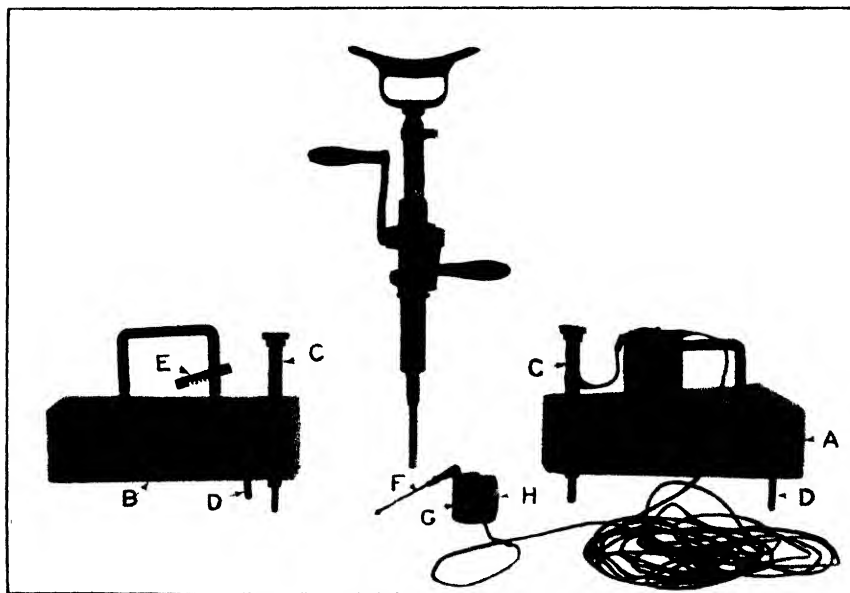
PLATE LXVI

Fig. 1.—Photograph of details of instrument for measuring wear of roads: *A*, Heavy mortar block; *B*, light mortar block; *C*, steel rod; *D*, level adjusting screws; *E*, adjusting rack; *F*, inside micrometer; *G*, steel bearing block; *H*, electric buzzer.

Fig. 2.—Instrument in use on concrete road.



1



2



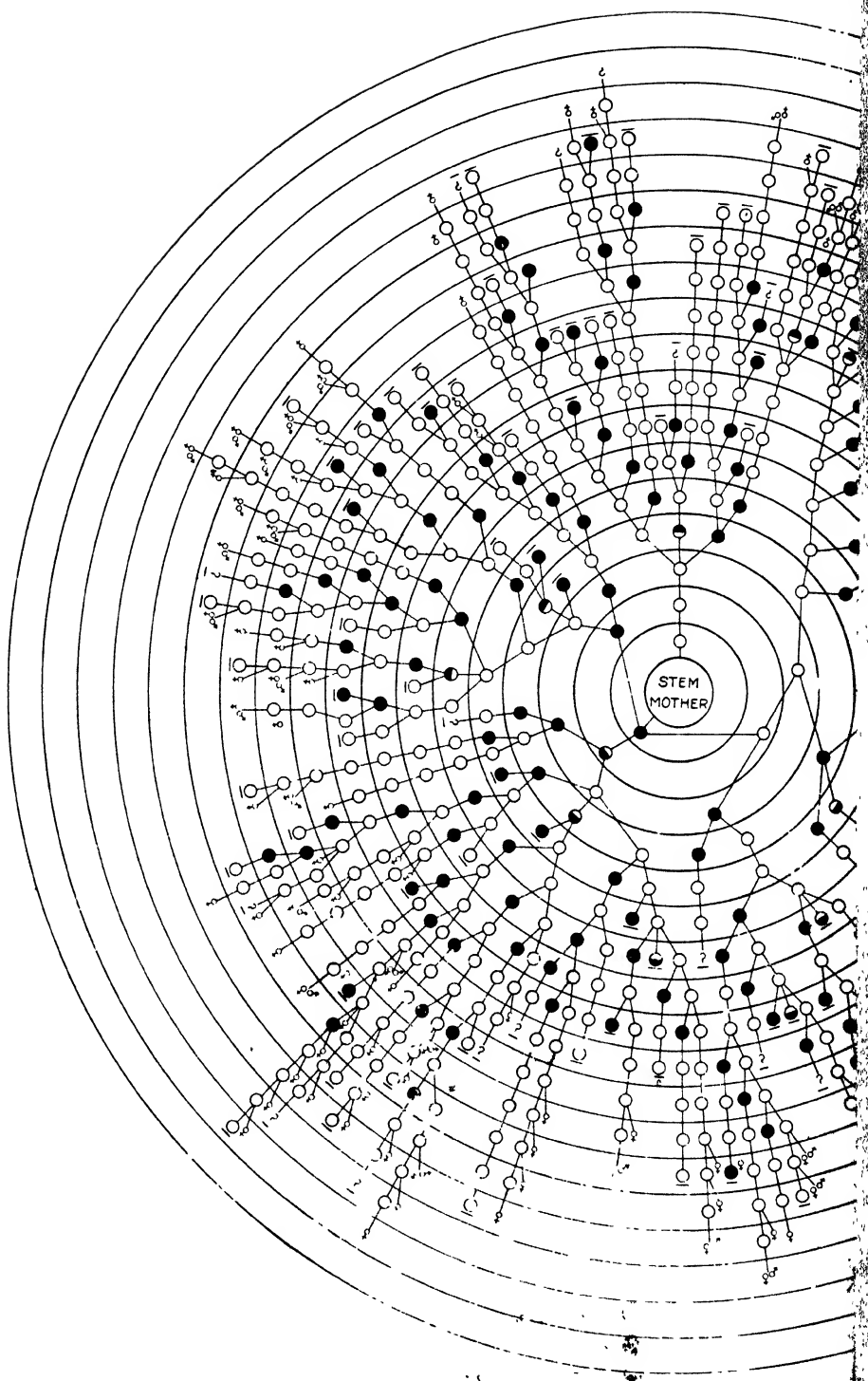


FIG. 4 - Genealogical diagram showing the forms and generations developing from one



most of the unfertilized females were observed to produce some sterile eggs, frequently laying the entire 6.

During the fall of 1914, eggs were first observed on the trees at Vienna, Va., on September 29. These were newly laid, being still yellow in color.

#### SUMMARY OF LIFE HISTORY

The life history of *Aphis pomi* may be briefly outlined as follows: The egg is laid upon the tender twigs of the apple, though occasionally it is laid upon the bark of the older twigs. It is light yellow when laid, but later changes to shining black. Development for a few days is very rapid, after which the egg rests for the winter. When the revolution of the embryo is completed in the spring, an increase in temperature will cause the egg to hatch. Before this revolution a high temperature only tends to destroy it. Early in April the egg hatches by a uniform splitting over the insect's head.

The stem mother is wingless and becomes mature in about 10 days. She produces summer forms, both winged and wingless, with the winged ones predominating. There are 9 to 17 generations of the summer forms at Vienna, Va. After the second generation the wingless forms always outnumber the others, but winged forms may occur in every generation. They become rare toward the end of the season. On the other hand, a wingless line may be carried from the stem mother to the egg. A third form, the intermediate, may occur throughout the summer.

The wingless sexes begin to appear about the 1st of September. They occur in all generations, from the eleventh to the nineteenth, inclusive, and probably also in the ninth and tenth.

The summer wingless forms and the oviparous females, which live longer than the males, remain on the trees at Vienna, Va., until the leaves drop, usually about the middle to the last of November.

Mating commences toward the close of September, one male usually serving more than one female. Both sexes feed. The oviparous female may lay infertile eggs if not reached by a male, and these eggs do not become black. The fertile egg develops to the resting stage before the first heavy frosts; otherwise it may be winterkilled and will not hatch to a stem mother the following spring.

#### GENEALOGICAL DIAGRAM

The accompanying diagram (fig. 4) shows the number of lines possible from one stem mother as indicated by the writers' breeding experiments. A line from each form reproduced in any given generation from known parents was carried until the sexual forms appeared. In some cases the lines indicated either died or were lost. The former are shown by a short transverse line (—) and the latter by (?). It will be seen from the chart that one direct wingless line was obtained from the stem mother

and that a similar wingless line was obtained from the winged offspring of the stem mother. No direct winged line was obtained, and in those where winged individuals were in some numbers intermediates usually occurred also. Each large circle in the chart represents a generation.

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## PLATE LXVII

Forms of *Aphis pomi*:

Fig. 1.—Winged viviparous female.

Fig. 2.—Male.

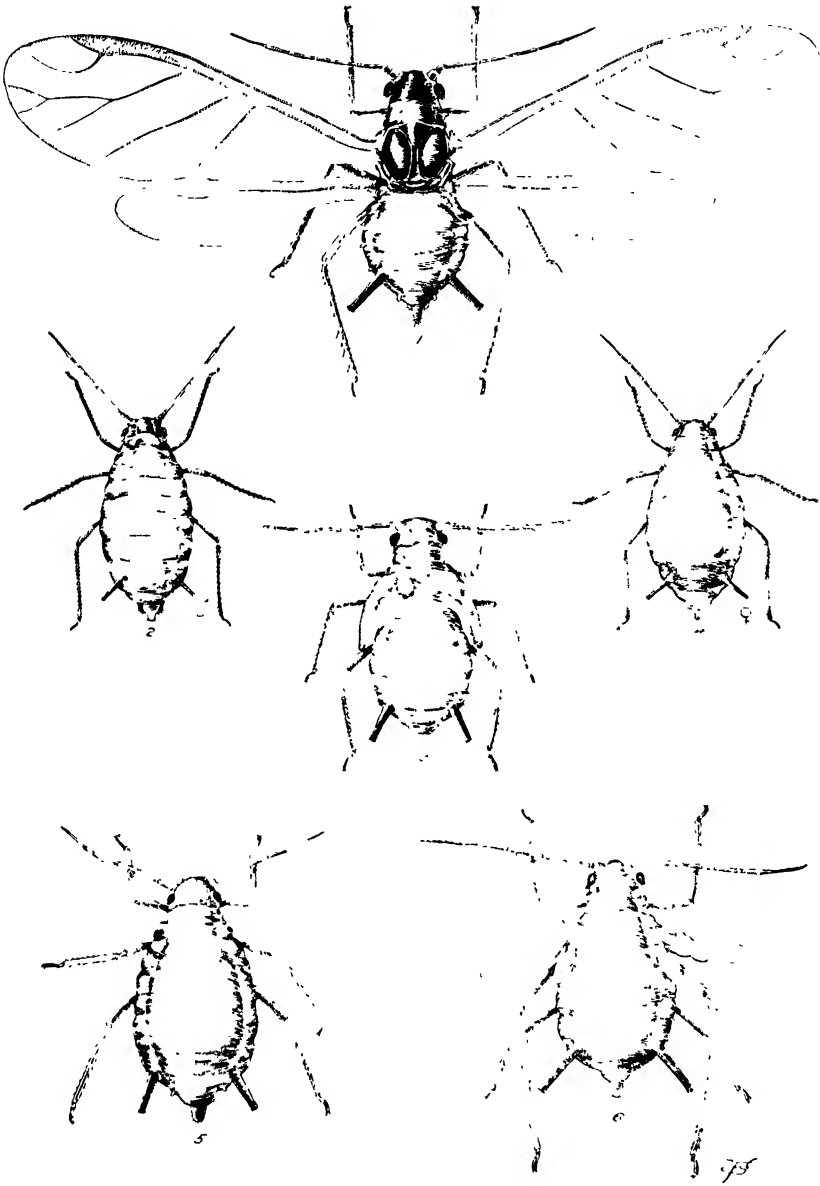
Fig. 3.—Pupa.

Fig. 4.—Oviparous female.

Fig. 5.—Wingless viviparous female.

Fig. 6.—Intermediate.

(994)





## PLATE LXVIII

### Embryology of *Aphis pomi*:

- Fig. 1.—Fertilized egg previous to formation of blastoderm.
- Fig. 2.—Fertilized egg showing formation of blastoderm.
- Fig. 3.—Unfertilized egg.
- Fig. 4.—Polar organ.
- Fig. 5.—Condition of embryo and polar organ at commencement of revolution.
- Fig. 6.—Yolk cell.
- Fig. 7.—Germ cell.

PLATE LXIX

Embryology of *Aphis pomi*:

Fig. 1.—Ovarian yolk before division.

Fig. 2.—Half of ovarian yolk shortly after "dumb-bell" formation.

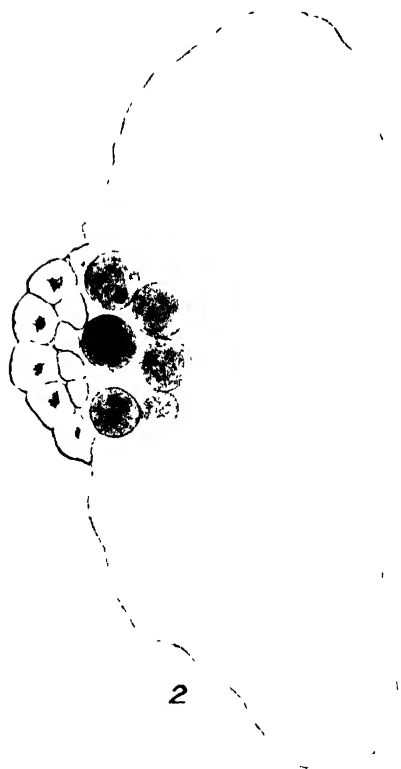




PLATE LXX

Embryology of *Aphis pomi*:

Fig. 1.—Half of ovarian yolk, end chambers forming.

Fig. 2.—Half of ovarian yolk, end chambers formed.



PLATE LXXI

Embryology of *Aphis pomi*:

Fig. 1.—Half of ovarian yolk, egg chambers forming; condition at time of hatching.

Fig. 2.—Thickening serosa accompanied by cells of polar organ.

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### MORPHOLOGY AND BIOLOGY OF THE GREEN APPLE APHIS

By A. C. BAKER and W. F. TURNER,

*Entomological Assistants, Deciduous Fruit Insect Investigations, Bureau of Entomology*

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#### INTRODUCTION

Owing to the abundance of the green apple aphid (*Aphis pomi* De Geer) at all times in most apple-growing regions and to the serious outbreaks of the species at different places and in different seasons, the writers were instructed to make a careful study of its life history. It was thought best to study the embryology of the insect in order, if possible, to explain the high mortality of the eggs in certain cases, their wintering condition, and, among other things, the most suitable time to attempt their destruction. Eggs were therefore taken during the winter of 1913-14 and again during that of 1914-15. With the opening of the season of 1914 generation experiments were begun at the deciduous fruit insect laboratory at Vienna, Va., and carried throughout the summer, fall, and early winter until the last sexes and eggs of the year were obtained. The material obtained from these experiments and the eggs in hand were studied and the manuscript prepared for publication during the winter of 1914-15.

During the summer the writers were assisted by Miss Dorothy Walton, and for three months by Miss Meta Neuman. These young ladies prepared the mounts of much of the summer material.

#### NAME OF THE SPECIES

The green apple aphid was first described by De Geer in 1773 (1, p. 53)<sup>1</sup> as follows:

*Aphis (pomi) flavo-virides, corniculis longioribus, pedibus antennisque nigrescentibus, Pomi.*

After giving this brief description De Geer enters upon a discussion of the insect, describing the different forms and giving interesting observations on the life history. For so early an account this is a very complete one and is much more valuable than many of those of more recent date.

In 1775 Fabricius (2, p. 737, no. 19) redescribed the species as follows:

*A. Pyri, mali.*

*Habitat sub pyri mali foliis.*

*Corpus viride, antennis pedibusque fuscis. Abdomen nec marginatum, nec plicatum. Anus terminator stylo nigro. Corniculi cylindrici, nigri. Variat corpore toto rufescente, pedibus fuscis et interdum pedibus lividis, geniculis fuscis.*

This name, *Aphis mali* Fab., was that by which the insect was commonly known until recent years. There seems, however, little reason for having adopted it, as Fabricius himself in 1794 (3, p. 216, no. 29) gives De Geer's insect as synonymous with his. He, however, uses his own name "*mali*" for the species and disregards De Geer's "*pomi*" altogether. "*Mali*," then, became the accepted name for the species. Unfortunately in this country the name "*mali*" was for many years applied to an entirely different species, now known as "*avenae* Fab.," under the impression that it was the apple insect of Fabricius. This error was first introduced into the literature of this country by Fitch (5, p. 65), and the same author later (6, p. 753-764; repr. p. 49-60) gave a very good description of *avenae*, under the name "*mali*." In this, however, he was only following European entomologists, such as Walker (4, p. 269), who used the name "*mali*" for an entirely distinct aphid.

Later writers followed in the same path, some, such as Buckton (7, p. 44, pl. 50), even confusing several species under the name. Sanderson (10, p. 191) used the name "*padi*" for this species in 1901. In more recent years De Geer's name has been given preference, and in this country the descriptions of Smith (9) and Sanderson (11, p. 130) have fixed the species to which it should be applied. The insect herein discussed must then be known as "*Aphis pomi* De Geer."

<sup>1</sup> Reference is made by number to "Literature cited," p. 992-993.

PLATE LXXIII

Embryology of *Aphis pomi*: Emerging nymph, showing egg burster.



1



2

## HISTORY AND DISTRIBUTION

Apparently the earliest record of the green apple aphid is the description by De Geer (1, p. 53), who states, in connection with this description, that he made rather extended observations of the species during the autumn of 1746. He also states that the insects were very abundant on the apple (*Malus* spp.) and often killed young trees. De Geer's observations were made in Sweden. Since the original description, many other European records have been made, and the species is now known to occur in every country of Europe and at least as far east as Turkestan in Asia. Many writers have reported it as being very injurious, particularly to young trees.

The unfortunate confusion of names makes it impossible to determine to which species the earlier records in this country really pertain. By previous writers *pomi* has been considered of much more recent occurrence in this country than the other apple species, *avenae*. This opinion, however, is not well founded. Although the descriptions given by Fitch (5, p. 65; 6, p. 753-764; repr. p. 49-60) prove that he considered *avenae* to be the true *mali*, an examination of the material from the Fitch collection shows that part of his insects were *avenae* and part of them *pomi*, even as they might be collected to-day by one not knowing the differences between the species. The specimens of *pomi* are marked "showing variations," which would indicate that, although Fitch noted the differences, he did not consider them of specific value. This shows *pomi* to have been located in this country nearly as early as we have any definite records. It was taken in Washington State in 1883 and in the District of Columbia in the same year. Williams collected it in St. Louis in 1894, and in all probability the forms referred to as *mali* by Cowen in 1895, in the bulletin by Gillette and Baker (8, p. 120) were *pomi*, since he observed both winged and wingless insects on the apple on August 23. It was present in Illinois in 1897, and no doubt was well distributed over the country much earlier than we have heretofore supposed.

In 1900 Smith (9) published a life history of this species. His first definite observations were made in 1897, and he first separated the species from the *mali* of American authors. In 1902 Sanderson (11, p. 130) published life-history notes on the species under the name "*pomi* De Geer."

It is known that this species occurs throughout the country wherever apples are grown. The accompanying map (fig. 1) merely shows definite localities from which we have records of the insect. It would indicate that the species is most abundant in the East. This, however, is not the case, since various observers in the West record it as occurring throughout their States. It appears to be particularly abundant in Colorado and the neighboring States.

*Aphis pomi* also occurs in Canada, being found from Nova Scotia to British Columbia. It has recently been recorded in the Kootenai and Okanagan districts of the latter Province.

Outside of Europe and North America few records of the species occur. It is present in Japan (18) and Dewar (12, p. 12) records it from Orange Free State.

It is rather remarkable that this aphid has not become even more widely spread, since it is typically a nursery species and in the egg state is easily transported on nursery stock.

Both in this country and in Europe *Aphis pomi* is usually abundant and particularly injurious at irregular intervals. Thus, in 1911 a severe outbreak occurred in Virginia, while in 1912 the species was very abun-

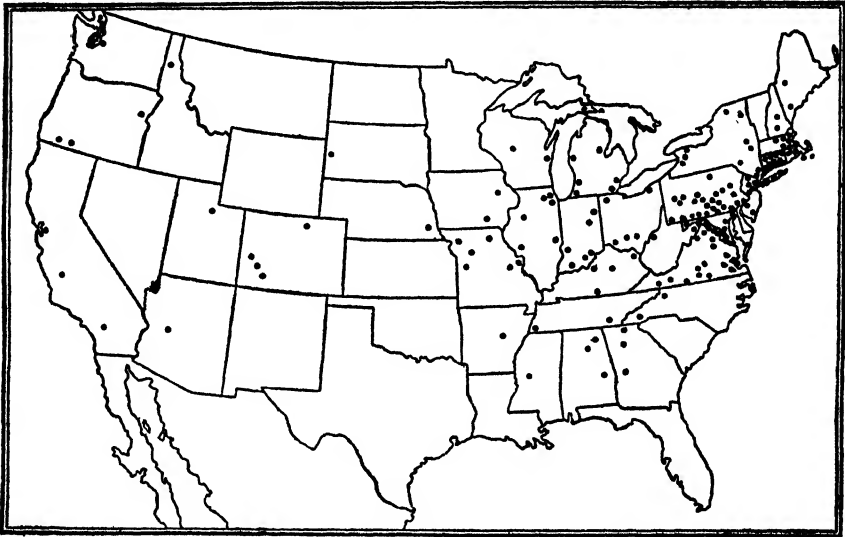


FIG. 1.—Map showing the localities in the United States from which the Bureau of Entomology has actual records of the green apple aphid (*Aphis pomi*).

dant in New England and New York. Similar phenomena have been noted from Russia. In some portions of this country, however, it seems to be always present and injurious. Gillette and Taylor (14) state that in Colorado "*A. pomi* is one of our very worst orchard enemies."

#### METHODS OF STUDY

EXPERIMENTS.—In initiating the experiments on which the following paper is based, twigs which bore eggs were collected at the time the eggs were beginning to hatch. These were kept under close observation. As soon as an egg hatched, the young stem mother was transferred to another twig kept in a vial of water. Although fairly satisfactory at first, this method of handling the food soon proved to be undesirable. Therefore there were substituted, first, dormant seedlings which had

been kept in a cellar all winter, and later young green apple seedlings grown in pots. In handling the dormant stock the tops were cut off, leaving a stem of 4 or 5 inches, and growth was started by keeping the roots in water for 8 or 10 days before planting.

The plants were covered by lantern-globe cages—inverted lantern globes with cheesecloth fastened over the bottom by a rubber band.

After the first two weeks all work was carried on in an insectary all four sides of which were made of fly screen. This duplicated normal conditions very closely, except that in most cases the direct rays of the sun could not reach the plants during the middle of the day.

In the actual handling of the insects it was found that it was much better to transfer adults than young, as this transfer of adults could be accomplished much more quickly and with greater safety, there being less danger of breaking the beak of the mature insects. Consequently several generations were reared, one after another, on one plant. This was also of great advantage in studying the effect of a prolonged use of good or poor food.

The usual custom in rearing aphides appears to be to raise the first born from the first born and the last from the last throughout the season. Since it was desired to raise young from both wingless and winged mothers in every case, this method proved to be impracticable. Moreover, the opinion was held—an opinion which has been confirmed by the past season's work—that the thorough study of a species can not be accomplished by such methods, because too few insects are reared. Consequently, as many insects as possible were carried to maturity, the number varying between a few to 60 or more for each experiment. The winged forms were transferred to new plants as pupæ. The wingless form was reared to maturity, and then all but from one to three insects were removed, these few being allowed to reproduce. All molts and specimens of insects from each generation were mounted for further study. At first each individual molt was mounted on a separate slide, but later, as their number grew into the thousands, this was impossible and a series of molts was placed on each slide. The total number of experiments conducted during the season was 1,720, with an approximate total of 15,000 insects. These insects, together with their molts, thus gave us for study nearly 75,000 individual forms of known lineage. The study of these forms has been tedious, but it has been a valuable adjunct to the actual breeding, furnishing many data which would otherwise have been unavailable.

It should be understood that, while the method above outlined was followed as closely as possible, it could not, from local causes, be applied in every case. However, it has been found to be very satisfactory and is believed to be a more efficient method for a thorough scientific study of the life history of aphides than any that has been seen recorded. •



**TECHNIQUE.**—For description, specimens were mounted in balsam in the usual way after having been dehydrated and cleared. Eggs were fixed with acetic-alcohol-sublimate solution, and after washing were preserved in 70 per cent alcohol. Those which had been preserved for some months gave better results on sectioning than did newly fixed material. Clearing was done in cedar oil, and sections from 5 to  $10\mu$  in thickness were cut; those  $8\mu$  gave the best results. Staining was done with Delafield's hematoxylin, orange G and picric acid, and Mayer's acid hemalum. Borax carmine was used for staining in toto.

## THE EGG

### DESCRIPTION

Size, 0.572 by 0.281 mm. Form oval, flattened on side next the bark; more or less covered with a glutinous substance which hardens with age. Color, glossy black.

The newly laid egg is not, as has been frequently stated for this species, yellowish green in color. It is a decided light-yellow, with rarely a slight tinge of green. It does, however, become somewhat greener during the change from yellow to black. This change is completed in the shade (insectary conditions) in from one to four days, usually a little over one.

The sterile egg can be easily separated from the fertile in that it is orange in color when laid. In one case such an egg finally turned to "ox blood," but this was the only example out of more than a hundred in which any color change took place before the egg began to shrivel up, at which time it sometimes became orange brown. This shriveling usually took place in about a week or 10 days after deposition.

### LOCATION ON TREE

The green apple aphid hibernates only in the egg stage. The eggs are laid in the fall on the smooth twigs, and especially on water sprouts. They are apparently never laid on the trunks of the trees, or even upon the branches. This is to be expected, since the females feed continuously during the oviposition period, and they would be unable to obtain their food through the thick bark (Pl. LXXV, fig. 2).

Unless the eggs are very abundant, they are usually deposited around and under the buds and in wounds in the bark. When abundant, however, they will be found scattered promiscuously over the twigs, and in some cases these will be entirely blackened with them. It is very interesting to note that in the winter of 1914 a careful survey of a large bearing orchard near Vienna, Va., revealed the presence of eggs only on trees in the south to west portion, and they were most abundant in the southwest corner of the orchard. These results were duplicated in an examination of a small orchard of 4-year-old trees on the laboratory grounds. More-

over, in both of these cases, and also in examinations of many isolated trees, the eggs were found to be much more abundant on the southwest sides of the trees.

The eggs adhere so tightly to the bark that great care is needed in removing them, and often this can not be done without breaking them. On downy twigs it is impossible to remove the eggs without also removing some of the hairs which adhere to them. Neither alcohol nor xylol will dissolve the adhesive or free these hairs from the egg.

#### EMBRYOLOGY

**GENERAL EMBRYOLOGY.**—The substance of the unfertilized egg is very clearly divided into two areas. The first, comprising nearly all the space included within the vitelline membrane, is filled with the food yolk, which consists of homogeneous granules enmeshed in a fine network of protoplasm. The second area, filled with smaller granules, which the writers are calling the "ovarian yolk," following Webster and Phillips (17, p.95), is rather spherical in shape and lies at the posterior pole. Surrounding these two bodies is a very narrow layer of peripheral protoplasm, the periplasm or "*Keimhaut blastem*" in which the blastoderm will form later. The egg is included within two envelopes, the vitelline membrane and the chorion.

At the time of deposition the fertilized egg appears like the sterile egg. In a very short time, however, the production of cleavage cells commences, and the formation of the blastoderm is initiated. This begins at the anterior pole and progresses most rapidly in that region, but in a short time covers the entire yolk, with the exception of the posterior end, where it lies in contact with the ovarian yolk. A portion of the cleavage cells do not migrate to the periphery, but remain in the yolk to become yolk cells.

Invagination commences by a thickening of the blastoderm in its area of contact with the ovarian yolk, brought about by the division of the blastoderm cells along this area.

At the end of about five days the germ band attains a condition in which it rests or hibernates till early spring. In this resting stage the embryo occupies a position in the center of the egg, with its cephalic portion directed toward the posterior pole. The posterior half of the abdominal region is flexed dorsad in such a manner as to include the ovarian yolk. Segmentation is well advanced, and the formation of the appendages has begun. The stomatodeum and proctodeum are present, while the formation of the mesenteron has begun. The genital rudiments are separated into two groups, although the ovarian yolk is not yet divided. At the posterior pole lies an organ composed of a single layer of cells surrounding a pear-shaped orange body without structural characters. This has been designated by Webster and Phillips (17, p. 98) as the "polar organ."

Development is resumed in the late winter or early spring (March 12 to 15, during 1914 and 1915, at Vienna, Va.). Growth is not resumed uniformly, even in a group of eggs on a single twig, some starting two or three days before the majority and a few not beginning to grow till nearly the end of March. This renewed development is accompanied by a movement of the embryo through the yolk toward the posterior pole till that portion of the amnion which lies above the head comes in contact with the serosa at its junction with the polar organ. The two envelopes then rupture at this point and the embryo revolves about its transverse axis to its definitive position.

From this time on development is rapid. The serosa contracts, and is invaginated and absorbed. The appendages are completed, the development of the digestive tract is consummated; nervous and muscular systems are perfected. Within a period of from five days to two weeks, depending apparently entirely upon temperature conditions, the insect is ready to hatch.

**OVARIAN YOLK.**—At the posterior pole of the egg there is situated an almost spherical, dark-staining body. This has been termed the secondary yolk by most writers, but has been designated the "ovarian yolk" by Webster and Phillips (17, p. 95). The writers are unable to follow the formation of this body, as no egg material earlier than those eggs deposited by the female was preserved. Tannreuther (13) studied its formation in *Melanoxanthrium salicis* L. He states that it is formed from the follicular nuclei of the oviduct wall, these dividing to form small vesicles which later unite and form common spherical masses. In the writer's earliest fertilized material (fertilized less than 24 hours) the ovarian yolk consists of a densely granular, almost spherical mass containing a number of large cells (Pl. LXVIII, fig. 7) which would correspond fairly well to the figures given by Tannreuther. At this time (Pl. LXVIII, fig. 1) the writers are unable to observe any cleavage cells within the body of the yolk, although there are at the anterior pole a number of dark-staining bodies well separated, but forming a dome-shaped structure conforming to the shape of the anterior part of the egg.

One thing is worthy of note in this connection. In unfertilized eggs, ranging in age from a few hours to 11 days, the ovarian yolk is a uniform, finely granular mass (Pl. LXVIII, fig. 3) without any of the large cells met with even in our earliest fertilized material. This leads to the belief that these bodies are associated with and appear only in connection with the beginning of growth. At the time the blastoderm is completely formed these bodies are present within the ovarian yolk and are surrounded by darker staining areas (Pl. LXVIII, fig. 2.) When the blastoderm is completely formed it covers the entire surface of the egg with the exception of the ovarian yolk, and invagination takes place about this yolk. (A single yolk cell is shown in Plate LXVIII, figure 6.)

It is thus carried to the interior of the egg with the developing germ band (Pl. LXIX, fig. 1). As the embryo develops, the ovarian yolk remains in connection with its posterior extremity, enlarges, and when this extremity becomes recurved, the yolk may be seen as a large, somewhat dumb-bell-shaped mass lying within the curve. At this time the large, deeply staining cells which form the end chambers of the ovaries are distinctly visible at its extremities. The remainder is a finely granular mass very similar in texture to that of the original ovarian yolk (Pl. LXIX, fig. 2). At a slightly later period the mass of the ovarian yolk becomes somewhat more enlarged in the heads of the dumb-bell at the expense of the "grip," and the end chambers are already forming (Pl. LXX, fig. 1). After the revolution of the embryo, the two heads of the dumb-bell-shaped yolk become separated, and it is henceforth represented by two large, slightly elongated masses, one on either side of the ventral portion of the body, the end chambers distinctly formed, and those on each side connected with one granular body of this ovarian yolk (Pl. LXX, fig. 2). In embryos almost ready to hatch, these two large granular bodies are still present, although more elongate than in the earlier stages. Some of the first egg chambers are now formed, and eggs may be noted within. The remainder of the reproductive organs are not yet developed (Pl. LXXI, fig. 1).

In the first instar of the stem mother these elongate granular bodies are still present. Webster and Phillips (17, p. 99) state that a group of cells which ultimately give rise to the generative organs separate off from the mesoderm during their "stage 6." The results of the present writers do not uphold this view. It seems more probable that these cells develop in the ovarian yolk, possibly from migrants, in the very early stages of growth, and that they are carried to the interior with this yolk at the time of invagination; that they here form two groups, one on either side of the ovarian yolk, which ultimately divides; and that these two masses of the ovarian yolk remain throughout embryonic development and assist in the formation of the reproductive system.

**POLAR ORGAN.**—Upon invagination the germ band leaves behind it, at the posterior pole of the egg, a group of large nucleated cells. This cell group has been recorded by Webster and Phillips (17, p. 98) as occurring in *Toxoptera graminum*, and was designated in their paper as the "polar organ." The writers have been unable to find any other reference in literature to the occurrence of such a body, either in the eggs of Aphididae or in those of any other insects.

The writers have not observed the genesis of this organ, but by the time the embryo has attained its "resting stage" it consists of a single layer of elongate cells surrounding a pear-shaped lumen (Pl. LXVIII, fig. 4). A large nucleus is present in the outer portion of each cell.

The lumen of this organ is occupied by a structureless yellow or orange-colored substance which extends by means of an elongated neck through an aperture in the chorion, thus opening upon the surface of the egg.

Webster and Phillips state that the yellow matter appears like a liquid. In *A. pomi* and in *A. avenae*, in which the organ is also present, it has more the appearance of a wax. Certainly it has a definite form which it maintains even when the surrounding cells are removed from it. The material is not affected by alcohol, xylol, or chloroform.

With the migration of the embryo to the surface and its revolution the cells of the polar organ are withdrawn, leaving the yellow body unchanged in form and still attached to the chorion. In one specimen which was in the late stages of development the yellow body was found inclosed by the anal portion of the embryo. Usually, however, it appears never to come in contact with the embryo; and when the latter hatches, it is left behind in the eggshell. The writers have been unable to find anything resembling it in any of the newly emerged insects.

**DORSAL BODY.**—With the resumption by the embryo of activities in the spring a change takes place in the cells of the polar organ. These flatten out, drawing away from the yellow mass as if the serosa were exerting an upward pull on them from all sides (Pl. LXVIII, fig. 5). Through the migration of the embryo the amnion finally comes in contact with the serosa at a point where the latter joins the cells of the polar organ, and both amnion and serosa rupture at this point.

As the embryo revolves, the serosa contracts until it lies as a thickened plate, the dorsal plate, near the anterior pole of the egg. In fact, in some cases the thickening takes place directly at the anterior pole, the plate moving later somewhat toward the posterior. During this contraction of the serosa it draws the cells of the polar organ after it, so that when the dorsal plate is formed, these lie as an irregular mass just posterior to the serosal cells (Pl. LXXI, fig. 2).

After the formation of the dorsal plate has been accomplished, this body commences to invaginate at its center, forming a tube which extends into the yolk ventrad, inclining slightly toward the posterior. This tube is formed of both the serosal cells and those which formerly constituted the polar organ. These cells can not now be distinguished from one another (Pl. LXXII, fig. 1).

This dorsal body soon separates itself entirely from the amnion and lies wholly immersed within the yolk in the form of a hollow sphere, one cell in thickness (Pl. LXXII, fig. 2). A little later this sphere breaks up and the cells disintegrate, probably being used as food by the embryo.

**RESTING STAGE.**—From the standpoint of life history the resting stage is one of the most interesting points in the embryology of this species. The embryo appears to be very seriously affected by changes of temperature at this time, or rather by sudden changes to temperatures

higher than those normally occurring out of doors. Several lots of eggs containing "resting" embryos were taken into the greenhouse at Vienna, Va., during the winter of 1915.<sup>1</sup> The first lot was taken on January 7 and other lots were taken at intervals of from one to two weeks until after growth was resumed. All the eggs in all lots died within two weeks. Over 50 per cent of all eggs placed in the greenhouse after the revolution of the embryo commenced, hatched normally.

It was at first thought that humidity might be a factor in this mortality, but the following experiment eliminated that. A very hairy twig which was well infested with eggs was cut in two. One half was placed in water, just as it was. The hairs acted as a wick, drawing the water to the top of the twig and keeping it and the eggs constantly moist. The base of the other twig was cleaned so that the water could not reach the hairs, and it consequently was dry. Both lots of eggs began to hatch on the same day. Moreover, hatching proceeded a little more rapidly on the dry than on the wet twig. It should be stated that the eggs used in this experiment had resumed growth before being taken into the greenhouse. These results are confirmed by the fact that no difficulty was experienced in hatching eggs taken into warm temperatures after the middle of March.

It will be seen that the temperature effect upon the egg at this period is rather a complicated matter. The activities of the embryo in the spring are apparently initiated by a general rise in temperature above the normal winter average. It seems probable also that these higher average temperatures must continue for some time for this species, since warm weather of two or three days' duration, occurring in January and February, does not appear to induce any growth whatever in the embryo. Certainly there is no appreciable difference between embryos collected just before such a period and those collected after it.

On the other hand, if the temperature affecting the eggs is artificially raised to greenhouse temperature (about 65° F.) at any time before the normal resumption of growth, the embryo dies. It is true that in certain instances some activity is induced, and embryos treated in this manner will be found to have developed somewhat, but in no case in these experiments did the revolution of the embryo occur.

From data of the writers it would seem that the embryos need to pass through a period of cold weather, perhaps even need to be subjected to freezing temperatures. This is indicated by the fact that in eggs laid early in the season the embryos had reached the resting stage and ceased growth for three weeks or a month before later eggs were deposited. Yet these later eggs in their turn developed normally to the resting stage.

The amount of low temperature needed by the insect is very uncertain. As suggested previously, it may be that a single freezing is sufficient, or

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<sup>1</sup> The average temperature in the greenhouse was about 65° F.

it is possible that continued cold weather, or a succession of freezings, is essential. In either case it seems probable that the embryo must have experienced a sufficient amount of low temperature long before spring and that it must thereafter continue to remain dormant till the proper average temperatures exist for its renewed activities. If, however, the embryos be subjected to temperatures well above the critical at any period before they have revolved, this change is fatal to them.

What this critical temperature is, can not be determined with any exactness from the data at hand. In 1915, from March 8 to 16, the period during which growth was resumed, the average temperature dropped to 34° F. only once, and it was below 36° only twice in the week. In 1914, however, the averages varied between 18° and 60° during what appears to have been the critical period, although from March 14 to 18, inclusive, it was above 34°. It seems probable that the critical temperature is close to 36°. Apparently, also, this critical temperature, or average temperatures a little above it, must continue for a period of some days, since frequently average temperatures higher than the critical occur for one or sometimes more days in January and February without affecting the insects.

It is interesting to note that eggs of *A. avenae* brought into the greenhouse during the winter hatched normally. Eggs of this species frequently hatch on the trees after warm spells of two or three days' duration in January and February; and while the writers have not as yet made a thorough study of the embryology of this species, yet during the winter they have taken several eggs in which the embryo had revolved.

These observations are of particular interest, since they undoubtedly explain the fact stated by several writers that a very low percentage—about 2 per cent, according to Gillette and Taylor (14, p. 24)—of the eggs of *A. pomi* hatch.

**HATCHING.**—The first eggs hatched in 1914 about April 8 and the last about April 25. At this time nearly all the buds showed some green and in many cases the tiny leaves were free from the bud scales. Since it is as immature stem mothers that this and corresponding species are usually treated with insecticides, it will be well to include here a comparison of their dates of hatching. In the spring of 1914, at Vienna, *A. avenae* commenced hatching on March 28. *A. malifoliae* and *A. pomi*, however, did not hatch until about April 8. A few eggs of *A. malifoliae* hatched before that date, and this would seem to indicate that the rosy apple aphid is perhaps slightly earlier than the green aphid. For all practical purposes, however, their hatching dates are the same, while that of *A. avenae* is very much earlier.

The young stem mother emerges from the egg head foremost, and the latter is always split evenly over the vertex of the insect. This is accom-

plished by means of a bladelike egg burster, which extends from the region of the trophic tubercle over the vertex and backward on the crown as far as the posterior margin of the eyes (Pl. LXXIII). This egg burster is often armed with one or two toothlike projections on its cutting edge. After the shell has been ruptured, the young, still within the membrane, protrudes for almost its entire length before the membrane ruptures. It is not uncommon to find insects which have reached this stage and died. They stand upward almost out of the shell, but still within the membrane. After the membrane has become ruptured and the insect has emerged, the former position of the egg burster is indicated by a suture-like marking extending over the vertex and crown and separating the two halves of the dark-colored cap met with in the stem mother of this species.

#### PLAN OF DESCRIPTIONS

It has been found by the study of the different instars that the easiest method for separating them is by the character of the antennæ. By measurements of these organs it is possible to determine immediately the instar of the form examined. In describing the different stages, therefore, in the earlier instars, measurements of the antennæ only are given, and these are followed by a complete description of the adult form. In the third instar of the summer forms those insects destined to become pupæ can be distinguished from those destined to become wingless only by the presence of the beginning of the wing pads. The measurements for both are the same. For the first two instars, therefore, only one description is given. The pupæ of the intermediate and that of the winged form are the same in every respect, and, therefore, only one description is given for these forms.

It is often important to know, immediately on their hatching from the egg, to what species apple aphides belong. We give here, therefore, measurements of the antennæ of the first-stage stem mothers of the more common apple-infesting species which are likely to be confused—viz, *pomi*, the green apple aphid, *avenae* (Pl. LXXIV, fig. 15, 18), the apple-grain aphid, and *malifoliae* (Pl. LXXIV, fig. 17), the rosy apple aphid. The adult stem mothers of these species could hardly be confused, on account of their different color characters, but the newly hatched insects are most easily and definitely separated by an examination of the antennæ.

The relative lengths of the proximal and distal portions of the fourth antennal segment in the different species are given in Table I, and an examination of these figures will enable one to separate the species easily.



TABLE I.—Lengths of third antennal segment and of proximal and distal portions of the fourth segment in *Aphis pomi*, *A. avenae*, and *A. malifoliae*

Species.	Segment III.	Segment IV base.	Unguis IV.
	Mm.	Mm.	Mm.
<i>Aphis pomi</i> .....	0.08 to 0.09	0.048 to 0.056	0.048 to 0.064
<i>Aphis avenae</i> .....	.08 to .096	.03 to .048	.08 to .104
<i>Aphis malifoliae</i> .....	.128	.048	.048 to .16

It will be noted that *A. pomi* has a much shorter unguis than either of the other species and this at once distinguishes it.

### STEM MOTHER

#### DESCRIPTION

**FIRST INSTAR.**—Morphological characters: Antennal segments (Pl. LXXIV, fig. 16) as follows: I, 0.032 mm.; II, 0.032 mm.; III, 0.08 to 0.09 mm.; IV, base 0.048 to 0.056 mm., unguis 0.048 to 0.064 mm.; segments III and IV imbricated and covered with a few stout spines, III armed with a distal sensorium, and IV with a sensory group at the base of the unguis composed of one large sensorium and several small ones. Eyes with 8 to 10 facets. Rostrum long. Cornicles short, thick, and rounded at the distal extremities. Cauda and anal plate rounded and densely setose. Legs with spinelike hairs.

Color characters: Body dark green (sometimes very dark) with appendages dusky; crown dusky to black with a median longitudinal uncolored suture-like stripe. Entire insect often slightly pruinose.

**SECOND INSTAR.**—Morphological characters: Antennal segments as follows: I, 0.032 mm.; II, 0.033 mm.; III, 0.08 mm.; IV, 0.048 to 0.064 mm.; V, base 0.048 to 0.064 mm, unguis 0.048 to 0.088 mm., usually about 0.075 mm.; segments III to V imbricated and with a few stout, spinelike hairs, IV with a distal sensorium similar to that on III of first instar, and V with the usual sensory group. Eyes with about 10 facets. Rostrum comparatively shorter than in first instar. Cornicles short and thick and rounded distad. Cauda and anal plate rounded and setose. Legs more slender than in preceding instar.

Color characters: Similar to the first instar, but lighter in color.

**THIRD INSTAR.**—Morphological characters: Antennæ much more slender than in the previous instars, with lengths as follows: I, 0.045 mm.; II, 0.042 to 0.045 mm.; III, 0.112 to 0.144 mm.; IV, 0.064 to 0.096 mm.; V, base 0.064 to 0.08 mm., unguis 0.088 to 0.112 mm., usually about 0.1 mm.; sensory characters as in previous instars. Eyes with over 20 facets; cornicles more elongate than in the other instars and not so rounded distad; cauda and anal plate rounded and setose.

Color characters: Similar to those of the previous instar.

**FOURTH INSTAR.**—Morphological characters: Antennæ fairly long and slender, with lengths as follows: I, 0.053 mm.; II, 0.048 mm.; III, 0.192 to 0.224 mm.; IV, 0.088 to 0.128 mm.; V, base 0.08 to 0.096 mm., unguis 0.112 to 0.128 mm.; segments III to V strongly imbricated, sensory characters as in other instars. Eyes with about 40 facets. Cornicles 0.153 mm. in length and imbricated. Cauda somewhat conical. Legs slender, tibiae somewhat curved, 0.571 mm. long.

Color characters: Approaching those of the adult form.

**FIFTH INSTAR (ADULT).**—Morphological characters: Antennæ (Pl. LXXIV, fig. 5) rather long and slender, with lengths as follows: I, 0.06 mm.; II, 0.056 mm.; III,

0.296 to 0.416 mm.; IV, 0.16 to 0.192 mm.; V, base 0.096 to 0.12 mm., unguis 0.16 to 0.184 mm.; segments III to V imbricated and armed with several prominent spinelike hairs, segment IV with a distal sensorium, and V with the usual group. Eyes prominent and with very many facets, ocular tubercles distinct; lateral thoracic tubercles prominent; abdominal tubercles not so prominent. Cornicles cylindric, tapering, imbricated and sometimes slightly flanged, 0.288 to 0.368 mm. in length. Cauda narrow, conical, or very slightly constricted toward its middle, densely setose and armed with a few long curved hairs. Anal plate rounded, setose, and hairy. Legs slender, hind tibiae 0.752 to 0.88 mm. long. Body quite globose, more so than that of summer form. Length, 1.92 mm.; width, 1.25 mm.

Color characters: General color green, somewhat darker than the summer forms; vertex and crown black; cornicles, cauda, and anal plates black, as are also the tarsi and the distal extremities of the tibiae, femora, and labium; eyes deep brown. The entire insect is sometimes covered with a bloom.

#### LENGTH OF NYMPHAL LIFE

The newly hatched stem mothers spend the first day wandering about over the twig on which they were born, doing little or no feeding. They finally settle on the tiny leaves or in some instances on buds in which the green of the leaves barely shows. From this time on they feed almost continuously, seldom changing their positions unless the food is very poor. In that case they may wander about on the twigs. Such insects, however, are very likely not to settle permanently nor to live to reproduce.

The duration of the first instar of stem mothers averages from 4 to 5 days; that of the next three, 6 days, the time being equally divided among the three. The total nymphal life thus averages from 10 to 11 days. That the first instar is longer than the three others, and also longer than the first instar of later generations, is due to the fact that the young stem mother loses one or more days in searching for suitable food. Prolonged cold spells would undoubtedly retard this development somewhat, but the insects can withstand short spells of severe weather with little or no apparent effect. Poor food conditions would probably check their growth also, but this factor is negligible, since the same conditions which induce hatching also cause the buds to burst, so that the food is practically always ready for the insects. Moreover, as stated previously, insects which fail to locate good food, and wander about, seldom reach maturity.

#### REPRODUCTION

The stem mothers begin to reproduce in about 24 hours after becoming adult. In the experiments of the writers the greatest number of young produced by one stem mother was 42, during a period of 10 days. In most of the species which have been carefully studied the average reproduction by stem mothers is greater than that by any of the succeeding generations. Considerable difficulty was experienced in handling this

form, many of the aphides leaving the plants and dying before the reproductive period was finished. Consequently 42 young is probably below the average under natural conditions.

The young are produced in groups of varying numbers and with unequal periods between the groups. In a general way an adult will produce a group one day and rest the next, but often the rest period will be longer and sometimes shorter. Individual mothers vary greatly in their rate of reproduction from the average rate. Some stem mothers ceased to reproduce for 2 or 3 days between some groups, while others never rested long. The greatest number of young produced in 24 hours was 9, one insect producing this number at two different times. In 4 days 22 were produced by one mother. The average daily production was 4.2.

#### LONGEVITY

The greatest length of life observed was 20 days. This is undoubtedly much below the true maximum and probably somewhat below the average. In the case recorded the insect produced young up to the last day.

The first stem mothers were observed on April 8 and the last May 6. Under natural conditions this period may perhaps be a little longer.

#### SUMMER FORMS

##### NUMBER OF FORMS

Beginning with the second generation and continuing until the sexes were produced, the writers found three adult forms to be present. The most abundant form was the wingless viviparous female. This occurred in every generation, and, with the exception of the second, always outnumbered the other forms present. It would often appear, in definite lines of descent, for several generations without being accompanied by winged insects. In fact, one purely wingless line was carried from the stem mother to the sexes, although in this case winged forms sometimes occurred as sisters or cousins.

On the other hand, the winged form was much more abundant than seems to be the case in most of the other species which have been studied. Winged insects were obtained in every generation from the second to the sixteenth, inclusive, although they became rare after the thirteenth generation.

The third form, the intermediate, occurred in 16 experiments, the first occurrence being in the third generation and the last in the twelfth.

In all, there were from 7 to 17 generations of the summer forms, the number depending upon whether the first or the last young were taken as mothers in each generation. In view of the fact that we have found winged forms to occur so commonly, it is difficult to understand how Smith (9) could have come to the conclusion that no winged insects

occurred after the third generation, an error in which he has been followed by many writers. He also states that only seven generations of the summer forms occur, another error which has been frequently quoted.

#### WINGLESS VIVIPAROUS FEMALE (PL. LXXVII, FIG. 5)

##### DESCRIPTION

**FIRST INSTAR.**—Morphological characters: Antennæ (Pl. LXXIV, fig. 4) as follows: I, 0.034 mm.; II, 0.036 mm.; III, 0.120 to 0.144 mm.; IV, base 0.064 mm., unguis 0.112 to 0.128 mm.; segments III and IV imbricated and armed with a few spinelike hairs, III with a distal sensorium, and IV with the usual sensory group at base of unguis. Eyes with 12 to 14 facets; cornicles short, thick and rounded distad; legs thick, hind tibiæ 0.239 mm. long.

**Color characters:** Color very variable from a light or dark green to yellowish. In some cases the insects are a golden yellow; the normal color is a medium green, never, however, as dark as the stem mother. Appendages dusky.

**SECOND INSTAR.**—Morphological characters: Antennæ (Pl. LXXIV, fig. 3) more slender than those of the other instars; lengths as follows: I, 0.045 mm.; II, 0.046 mm.; III, 0.112 to 0.152 mm.; IV, 0.08 to 0.096 mm.; V, base 0.056 to 0.08 mm., unguis 0.144 to 0.176 mm.; segments III to V imbricated and with a few spines, IV with distal sensorium similar to that on III of first instar, and VI with the usual group. Eyes with 28 to 30 facets. Cornicles rounded at the distal extremity, thick and imbricated. Legs stout and covered with spinelike hairs, hind tibiæ 0.320 to 0.384 mm. in length. Cauda and anal plate setose, cauda somewhat conical.

**Color characters:** Similar to those of first instar.

**THIRD INSTAR.**—Morphological characters: Antennæ (Pl. LXXIV, fig. 2) rather long and slender; lengths as follows: I, 0.048 mm.; II, 0.051 mm.; III, 0.192 to 0.248 mm.; IV, 0.112 to 0.144 mm.; V, base 0.08 to 0.096 mm., unguis 0.2 to 0.232 mm.; segments III to V imbricated and bearing a few spines, the base of V strongly but regularly imbricated but the unguis quite regularly, so giving the appearance of almost complete rings; sensoria as in previous instar. Eyes with 38 to 40 facets. Cornicles slightly rounded at distal extremity, but not nearly as much as in previous instars, length about 0.188 mm. Legs more slender than in previous instars, hind tibiæ 0.448 to 0.054 mm. long. Cauda and anal plate setose, cauda bluntly conical.

**Color characters:** Similar to those of first instar.

**FOURTH INSTAR.**—Morphological characters: Antennæ (Pl. LXXIV, fig. 10) long and slender; lengths as follows: I, 0.62 mm.; II, 0.06 mm.; III, 0.144 to 0.192 mm.; IV, 0.134 to 0.176 mm.; V, 0.152 to 0.192 mm.; VI, base 0.088 to 0.112 mm., unguis 0.248 to 0.304 mm.; segments III to VI distinctly imbricated and armed with a few prominent hairs, segment V with a distal sensorium (the original III of first instar now represents III, IV, and V). Eyes with about 58 facets. Cornicles rather slender, compared with the earlier ones, cylindric, imbricated, and about 0.264 mm. long. Hind tibiæ 0.672 mm. long. Cauda and anal plate setose, anal plate rounded, cauda bluntly conical.

**Color characters:** Similar to those of first instar. The appendages are here partly turned to the black color met in the adult form. The cornicles blacken from the distal extremity proximad.

**FIFTH INSTAR (ADULT).**—Morphological characters: Antennæ (Pl. LXXIV, fig. 1) long and slender compared with the early instars, but short compared with the body; lengths as follows: I, 0.064 mm.; II, 0.063 mm.; III, 0.224 to 0.320 mm.; IV, 0.176 to 0.240 mm.; V, 0.176 to 0.232 mm.; VI, base 0.104 to 0.128 mm., unguis 0.28 to 0.32 mm.; segments III to VI imbricated and with a few stout hairs; sensoria as in fourth instar. Vertex slightly rounded. Prothorax with a prominent tubercle on each side.

Abdomen with five distinct tubercles on each side, the one pair caudad of the cornicles and the most cephalic pair about equal in size and larger than the three median pairs. Cornicles (Pl. LXXIV, fig. 12) subcylindric, largest at the base, tapering slightly distad, slightly flanged at the tip and strongly imbricated, 0.398 mm. in length. Anal plate rounded, setose, and armed with about a dozen long curved hairs. Cauda (Pl. LXXIV, fig. 19) elongate, rounded distad, sometimes slightly constricted in the middle, setose, and armed on each side with about five long, curved hairs; length, about 0.176 mm. Legs slender, hairy, particularly the tibiae; length of hind tibia, 0.837 mm.; hind tarsi, 0.112 mm.; length of insect from vertex to tip of cauda, 2.56 mm.

Color characters: General color very variable, from a light green to a very dark green. Head orange-yellow, sometimes with a purplish cast. This orange-yellow head is in many specimens much more pronounced than in others. Thorax similar to the head in color, shading off into yellowish green at the abdomen. Both head and thorax covered with a slight bloom. Abdomen light green. Antennae yellowish, dark toward the tip; tarsi, cornicles, cauda, anal plate, distal extremities of femora, and proximal and distal extremities of the tibiae black. Labium tipped with black. In specimens which have not been well supplied with food and which consequently are much stunted in growth, the colors are much deeper, the green being very dark over the entire body, whereas in well-fed, large specimens the color is light green. Late fall specimens which are exposed to low temperatures have a brownish cast.

#### OCCURRENCE

As stated previously, this was by far the most common form occurring during the summer. Moreover, in so far as the actual propagation of the species is concerned, it is the only summer form necessary, since we were able, without difficulty, to carry insects from the stem mother to the sexes without the intervention of a single winged individual. For the spread and consequently the greatest development of the species, winged summer forms seem necessary, since at the present time it has no other natural mode of becoming wholly disseminated. In nurseries the wingless insects may travel from tree to tree in the rows, and trees bearing eggs may be shipped to different parts of the world. Such dissemination, however, would be of little avail to a purely wingless species, as compared to one containing winged forms, since its attack thereafter would be confined to trees on which it was shipped, or at most to a few surrounding trees.

#### LENGTH OF NYMPHAL LIFE

The average duration of the nymphal period in this form was 7 to 8 days, the time being equally divided between the four stages. During the hot weather occurring in the last of June and first of July this period was shortened to 6 days, and in one instance an insect commenced reproduction when only 5 days old. On the other hand, with the beginning of cooler weather in the late summer the period exceeded this average. About September 1 the time occupied by the nymphal stages was from 8 to 9 days. This period gradually increased in length till the last of September, at which time it covered 11 days. During the month of

September the temperature dropped below 50° F. several times, reaching 37° in one instance. These extreme temperatures were of short duration, however, and the mean was never below 50°. By the end of October the nymph required 12 to 14 days to attain the mature condition. At times during this month the temperature averaged between 53.5° and 59° for periods of 24 to 36 hours. During such periods very little feeding or growth took place. The insects would stand perfectly motionless. Mechanical stimulus with a needle merely induced slight movements of one or two legs. Moreover, it required considerable time for the insects to recover from such conditions, and often maximum temperatures of 65° to 70° would not cause a resumption of active feeding.

The difficulty of exactly correlating the rate of growth with temperature conditions is greatly increased by the fact that the condition of the food supply was as great or even a greater factor in determining this rate of growth. This factor can only be appreciated, however, in marked cases. Usually the observer is unable to determine which of two plants offers the insects the best food, and consequently is unable to gauge the proper values of the two factors. The effect of the food condition is taken up more fully in another place (p. 983).

#### REPRODUCTION

As in the stem mothers, the wingless viviparous females begin reproduction about 24 hours after becoming mature. In fact, this condition obtained for all viviparous females, whatever the form.

The average reproduction varies greatly during the season and the writers find that their figures separate into three well-defined groups: First, reproduction by the summer forms born before July 1, and reproducing by July 6; second, reproduction by forms born between July 1 and September 1, beginning to reproduce between July 6 and September 10; third, forms born after September 1, commencing reproduction after September 10. Eighty wingless individuals in the first group produced an average of 55.4 young per insect; 113 wingless individuals in the second group averaged 30.9 young, while in the third group 24 wingless individuals averaged 12.1 young apiece. The last mothers of the season produced only from 1 to 4 or 6 young. The average reproduction per insect per day during the first period was 2.95, during the second 1.92, and during the third 0.83.

For the entire season the average per wingless insect was 37.5, and the daily average was 2.22. The greatest number of young produced by one individual was 133, while the maximum reproduction for one day was 16+, one insect producing 64 young in 4 days.

The rate of reproduction was very irregular. In some cases the majority of young were produced early in the life of the adult. In others comparatively few were produced during the first few days and then large

numbers were brought forth. Some insects bore numerous young daily till death; with others the production decreased gradually to that point; while in a third class the insects lived from 3 to 44 days after reproduction ceased, the longer period occurring in the fall, October and November. During the summer the longest period was 13 days. In one remarkable case an insect born on September 29 produced 10 young in 13 days (October 13 to 26). It then ceased to reproduce till December 5 (40 days), when it bore one young and died.

#### LONGEVITY

The average total length of life for the entire season was 30.9 days. This average is only for insects which reached maturity. Many died while still nymphs. The greatest length of life attained by one insect during the summer was 48 days. In the fall the average period was longer than in the summer, and one insect lived 68 days.

Wingless viviparous females were present on the trees until within less than a week from the time of the last appearance of oviparous females—i. e., during the fall of 1914 until after November 20. In the cages one insect was alive on December 22.

#### HARDINESS

A rather interesting note was made during the fall on the effect of low temperature on the activities of this species. On December 22 an examination of about 50 insects, including wingless viviparous females and oviparous females, showed all the insects to be perfectly motionless, except one viviparous female. This insect moved both legs and antennæ when irritated slightly with a camel's-hair brush. The temperature at the time the observations were made was 34° F. and had remained constant for about 2 hours. For the 12 hours previous the temperature had been 30° F. or less. This would indicate that at least in individual cases the developmental or physiological zero for this species is quite low.

#### WINGED VIVIPAROUS FEMALE (PL. LXVII, FIG. 1)

##### DESCRIPTION

**FIRST, SECOND, AND THIRD INSTARS.**—In the first and second instars these insects are identical in form with those producing wingless adults. In the third instar the measurements are the same for those given under third instar wingless female, but beginnings of wing pads are present.

**FOURTH INSTAR (PUPA)** (PL. LXVII, FIG. 3).—The pupæ producing intermediates and those producing winged forms are identical, as follows:

**Morphological characters:** Antennæ as follows: I, 0.06 mm.; II, 0.06 mm.; III, 0.176 to 0.256 mm.; IV, 0.128 to 0.176 mm.; V, 0.128 mm.; VI, base 0.80 to 0.112 mm., unguis 0.216 to 0.28 mm.; sensoria, imbrications, etc., as in the wingless form. Vertex rounded, with a slight median indentation. Eyes prominent, with a large number of facets; ocular tubercles distinct and with usually three lenses. Thoracic and abdom-

anal sutures as in the wingless form. Wing pads prominent, extending somewhat caudad of the hind coxae. Cornicles subcylindric, imbricated, slightly flanged; length, 0.168 to 0.376 mm. Legs slender, hairy, hind tibiae 0.504 to 0.64 mm. long. Anal plate rounded, setose and armed with hairs. Cauda (Pl. LXXIV, fig. 21) conical, not as in the adult form, setose, and armed with many long, curved hairs. Length from vertex to tip of cauda, about 2.6 mm.

Color characters: General color greenish; head and thorax orange-yellow with a rosy bloom, the reddish appearance of this increasing with age. Abdomen yellow-green. Antennae yellowish, with the distal segments dusky. Wing pads brown, with black costal margins. Eyes, tip of labium, tarsi, and distal extremities of tibiae and tarsi black; cauda lighter than abdomen, not black as in adult. Area between cornicles darker green than the rest of the abdomen. In some cases the margins of the thorax are light-straw color, almost white, venter usually lighter than dorsum.

FIFTH INSTAR (ADULT).—There is no distinct spring or fall migrant in this species. All the winged individuals occurring throughout the spring, summer, and fall have the same characters and are identical, except for variations bearing no relation to season.

Morphological characters: Antennae (Pl. LXXIV, fig. 7) as follows: I, 0.064 mm.; II, 0.063 mm.; III, 0.192 to 0.312 mm.; IV, 0.144 to 0.288 mm.; V, 0.144 to 0.224 mm.; VI, base 0.096 to 0.128 mm., unguis 0.288 to 0.344 mm., segments III to VI imbricated and armed with a few hairs, III with a row of usually 6 circular sensoria (range 4 to 9). These sensoria form an even row along the segment and are of about the same diameter as the segment. They have a distinct double rim. Segment IV often without sensoria, although on some specimens there are as many as 3 on this segment near its distal extremity. Sometimes one antenna has sensoria here and the other none. Segment V with a distal sensorium, and VI with the usual group at the base of the unguis. Vertex slightly rounded, median ocellus protruding, lateral ocelli very close to the compound eyes; these eyes large and showing with distinct tubercles. Thoracic and abdominal tubercles as described for the wingless form. Wings with delicate veins; forewing with the media normally twice branched, but not uncommonly with it only once branched and in rare cases (approaching the intermediate) this represented by one vein only. Cornicles (Pl. LXXIV, fig. 11) subcylindric, tapering toward the tip, imbricated and slightly flanged; length, 0.192 to 0.352 mm. Anal plate rounded, setose, and armed with a number of long, curved hairs. Cauda elongate, slightly constricted in the middle, rounded at the tip, densely setose, and armed on each side with about 5 long, curved hairs. Legs slender; hind tibiae 0.56 to 0.992 mm. long. Length from vertex to tip of cauda, about 2.5 mm.

Color characters: Head and thorax shining black, sutures yellowish; antennae straw color at base, dark, almost black distad; eyes black; legs yellowish, with the distal extremities of the femora, the distal and proximal extremities of the tibiae, and the tarsi black. Abdomen yellow-green, with the margins and a longitudinal median stripe darker green. Cauda and anal plate black. Labrum straw color, with tip dusky or black. Wings hyaline, veins brown, stigma smoky.

Most of the winged forms had the abdomen uniform green, but with the second winged generation another form appeared. The color of this is as follows: Head and thorax black, similar to the first winged generation; veins and stigma dark; abdomen unlike the uniform pea green of the first winged generation, but much darker, with a median longitudinal stripe of still darker green; margin of the abdomen on each side with a row of 5 or 6 dark patches; other characters as in first winged generation.

The color characters of this winged generation may have had something to do with the confusion of *A. pomi* and *A. avenae*, as the color characters of the two are quite similar.



## CAUSE OF PRODUCTION

The theory has been frequently advanced that the production of winged forms during the summer is due to a lack of sufficient nourishment for the insects. In some cases the wording of this theory is modified by the statement that winged forms appear on plants which are very heavily infested. The writers' results are a flat contradiction of this theory for this species.

As has been stated previously, in handling the insects the writers always transferred the mothers to new plants, rather than the progeny. In this way several consecutive generations were reared on one plant. Thus the effect of poor or good food would be accentuated. Yet the winged forms were never obtained in series of small, poorly fed insects, but occurred frequently in well-nourished series.

It should be stated that these results are not based on deliberate experiments to obtain data on this point. Notes were made simply because of a very evident abnormality in size and rapidity of development, correlated with a lack or an abundance of food. Later, in studying the notes, it was found that the large, well-fed insects developed rapidly and often produced winged forms, while many of the small, starved aphides produced only wingless progeny. Moreover, none of the plants was heavily infested, so the production of winged aphides can not be correlated with that condition.

In addition to the foregoing data, it was found that those winged insects produced during the summer months showed little or no inclination to leave the plants on which they were produced. This would at once disprove the theory that these winged forms are produced when the insect meets adverse food conditions in order to carry it to better food.

Other writers have maintained that the winged insects were produced as the result of an abundance of certain chemicals in the soil. The writers' work would not certainly contradict this theory. Still, the fact that the soil used was mixed in large batches and that winged forms were produced on some of the plants, while other plants raised in soil from the same batch bore only wingless forms would seem to cast considerable doubt on its truth. It is also very difficult to understand how the occurrence of such a form as an intermediate could be made to conform to this theory.

The writers' results, deduced from very full notes on the life history of this aphid, lead to the belief that much of the evidence given in favor of these theories is based on insufficient data.

It seems much more probable, especially in view of the quite frequent occurrence of such a form as the intermediate, that the production of this winged form during the summer is merely a reversion from the wingless to the more primitive aphid form. As such it is doubtful whether food conditions have anything whatever to do with the matter.

## OCCURRENCE

Although, as has already been stated, this form is not necessary for the successful propagation of the species, it occurs quite commonly throughout the greater part of the summer. In the second generation the winged form outnumbers the wingless, although the writers were unable to determine the exact proportion. Thereafter winged insects are always less abundant than wingless.

This form occurred, in the writers' experiments, in every generation from the second to the fifteenth, inclusive. It was of very rare occurrence, however, after the thirteenth generation. In the complete life-history diagram (fig. 4) it occurred 149 times, each occurrence representing a different combination of the two factors, form and generation, among the ancestors.

In the field winged forms were apparently present in small numbers all summer. Definite observations were made on several days during July and August. In all cases migrants were found in every colony of any considerable size.

It is very interesting to note that in only 18 cases were winged forms produced by winged mothers, and in only one case did three winged generations occur in succession.

The last winged insects were born on September 9; none were found after October, either in the experiments or on the trees.

## LENGTH OF NYMPHAL LIFE

The immature stages of this form covered a period of two more days than did the same stages of the wingless form. This extra time was occupied in the pupal instar, the three earlier stages requiring the same amount of time as the corresponding stages of the wingless form.

## REPRODUCTION

Dividing the season into periods similar to those used in the discussion of the wingless reproduction, the writers obtain the following figures: The average reproduction by 29 winged insects during the first period (to July 1) was 50.1 per mother; that of 25 insects in the second period (July 1 to September 1), 25.4 per mother. Very few winged insects occurred during the third period, and the writers have no complete records of progeny from any individuals. During the first period the average per insect per day was 2.92. During the second period it was 2.04.

The seasonal average production per insect was 39, while the daily average was 2.62. The greatest number produced in one day was 6, and the maximum number of young produced by one individual was 120 (in 21 days). The average length of the reproductive period for the entire season was 20.75 days.

## LONGEVITY

The longest total life recorded for an individual of this form was 42 days.

## FLIGHT

A large number of migrants of the second generation were reared on some small apple trees in the laboratory. These insects, on becoming adult, were very active, and several hundred were taken on the windows of the room in which they were confined. They were to a marked degree negatively geotropic. This was well illustrated by the fact that as many as 25 could be kept safely in a small open vial by simply holding it upside down. Almost without exception migrants transferred to new plants settled readily and made no attempt to fly farther. They were very likely to fly from the brush, however, during the process of transfer.

In the case of the later winged forms no such tendency toward flight was observed. In no case were winged aphides observed which had left the plants and clustered on the sides and tops of the cages, unless the plants were so nearly dead that the wingless forms also left them. Moreover, no particular caution was necessary in transferring them from one plant to another, since they showed no inclination toward flight. This would seem to indicate that the winged forms of the second generation alone correspond to the spring migrants of species with a definite alternation of hosts.

## INTERMEDIATE FORM (PL. LXXVII, FIG. 6)

## DESCRIPTION

Morphological characters: Antennae (Pl. LXXIV, fig. 6) as follows: I, 0.064 mm.; II, 0.064 mm.; III, 0.28 to 0.34 mm.; IV, 0.16 to 0.24 mm.; V, 0.144 to 0.208 mm.; VI, base 0.096 to 0.12 mm., unguis 0.176 to 0.328 mm. Antennal segments armed as in wingless individuals, with the exception of segment III, which is armed with unequal sized sensoria, varying from 4 to 6 in number. Vertex rounded; eyes with ocular tubercles present; ocelli absent, even from specimens with nearly half-size wing rudiments. Thorax and abdomen with tubercles as in the wingless form. Thorax not showing the distinct "corseletta" of the winged form, but indicating a series in these forms from the winged to the wingless condition. Wings of winged form represented here by reduced wings of about half the normal size, through gradations in different individuals until mere folds of the skin are seen. Cornicles subcylindric, tapering distad, imbricated, and slightly flanged; length, 0.272 to 0.496 mm. Anal plate rounded, setose, and armed with long hairs. Cauda elongate, slightly constricted in the middle, rounded at the tip, densely setose, and armed with five or six long curved hairs on each side. Leg slender, hairy; hind tibia 0.608 to 0.896 mm. long. Length of insect from vertex to tip of cauda, about 2.5 mm., but with much variation.

In general outline the intermediate conforms much more closely to the wingless insect than to the pupa, being plump and of regular outline without having the thorax sharply delineated.

Color characters: In color characters this form resembles the wingless female very closely. In most specimens the rudiments of the wings are of a light green color,

nearly the color of the abdomen, while in some others they are a dusky gray. In specimens that have wings as large as the normal hind wing of the winged form, these wings are transparent like those of the winged. In other color characters this form resembles the wingless female.

#### COMPARISON WITH USUAL FORMS

Up to and through the pupal stage these insects appear to be identical with the immature stages of the true winged aphides. In fact, the writers are not able to distinguish the pupal molts from which intermediates emerged from those shed by the winged insects.

The adults, however, more closely resemble the wingless individuals than the winged, in general bodily outline. They lose the "corseletta" of the thorax, which latter at the same time becomes less distinctly differentiated from the abdomen, conforming quite closely to the wingless form. The darker color is also lost, the head and thorax being concolorous with the abdomen.

Two indications of the winged character are retained, however. These insects bear rudiments of wings, varying from wings of nearly half size, with indications of some of the veins, to tiny pads which are hardly more than wrinkles of the skin. Also the antennæ of this form bear, on the third segment, sensoria like those of the winged insects, which are absent in the wingless form. These, however, are not normal, in that usually the entire six are not present, the numbers on the two antennæ vary, and the sensoria are not of uniform size, very few being as large as the normal ones.

One other interesting point is that the dorsoventral muscles of the thorax, which are developed in connection with flight, are very much reduced in all specimens and the longitudinal thoracic muscles are reduced in varying degrees, the amount of reduction in both cases coordinating quite closely with the reduction exhibited by the wings. The writers (19) believe these intermediates to be variants between the winged and wingless forms, and of perfectly normal occurrence, illustrating the steps by which the wingless condition has been attained in the Aphididae.

#### OCCURRENCE

Intermediates were of rather common occurrence, being observed, as stated above, in 16 experiments. In all, 31 individuals were found.

#### LENGTH OF NYMPHAL LIFE

The nymphal period was of the same length as that of the winged form. In fact it was impossible to distinguish between the two forms in any manner, until the adult condition was attained.

## REPRODUCTION

Reproduction was perfectly normal. Both wingless and winged forms were produced, though the percentage of wingless forms was a little greater than by the wingless mothers. Three adults produced 81 young, an average of 27. This is much below the average for the other forms, but only 3 insects were used, and there is nothing to indicate that, normally, this form would not produce at least as many young as the winged mothers. The average daily reproduction was 2.13 for these three individuals, this being somewhat less than that of either of the other forms. Here, again, however, the small number of mothers detracts from the comparative value of the figures.

## LONGEVITY

The average length of life for these three insects was 24.3 days, one living 27 days.

## COMPARISON OF THE THREE FORMS

## NYMPHAL STAGES

All three forms agree in having four immature stages, the first three existing for equal periods, while the last stage is about two days longer in the winged individuals and intermediates than in the wingless ones.

## REPRODUCTION

Table II gives a comparison of the reproductive activities of the three forms.

TABLE II.—Comparison of the reproductive activities of the three summer forms of *Aphis pomi*

Form.	Number of insects.	Average per insect.				Average per day.				Maximum per insect.	Maximum per day.
		First period.	Second period.	Third period.	Season.	First period.	Second period.	Third period.	Season.		
Wingless.....	80	55.4	.....	.....	.....	2.95	.....	.....	.....	.....	.....
Do.....	113	.....	30.9	.....	.....	.....	1.92	.....	.....	.....	.....
Do.....	23	.....	.....	12.1	.....	.....	.....	0.83	.....	.....	.....
Do.....	217	.....	.....	.....	37.5	.....	.....	.....	2.22	133	16+
Winged.....	29	50.1	.....	.....	.....	2.92	.....	.....	.....	.....	.....
Do.....	25	.....	25.4	.....	.....	.....	2.04	.....	.....	.....	.....
Do.....	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do.....	54	.....	.....	.....	39	.....	.....	.....	2.62	120	6
Intermediate.	3	.....	.....	.....	24	.....	.....	.....	2.13	27	5

It will be noticed that a comparison of the figures for the entire season gives the winged form a larger average reproduction per insect than the wingless. This is because no winged individuals occurred during the third period when the number of young produced was very low. Eliminating this factor we find that for the first two periods the average for wingless insects was 43+ young, while for the winged aphides it was only 37.7. Unweighted averages have been used here, since it is desired to compare merely the production by the two forms under similar conditions, and the fact that wingless insects were so abundant during the second, or poorer, period would make the use of weighted averages unfair.

#### PRODUCTION OF SEXES

Both wingless and winged viviparous females may, in addition to producing viviparous females, produce the sexes. However, the wingless individuals are much more commonly sexuparous than the winged insects, since sexes were reared from only three individuals of the latter form.

#### DIMORPHIC REPRODUCTION

No exact data are available on which to base statements as to the prevalence of dimorphic reproduction—the production of two different forms by one mother. Nevertheless, enough data are at hand to show that it is of very frequent occurrence during the summer and may even be the rule. In several cases wingless, winged, and intermediate mothers produced both wingless and winged offspring. In many cases the first young produced were all wingless, while later progeny were winged; but this was not always true, since the very last young were sometimes wingless.

In a very few cases wingless mothers produced both viviparous and oviparous females, and in one or two instances both males and oviparous females. Again, in a few cases it was possible to determine that one mother produced both oviparous females and males, while in one instance a single viviparous insect produced viviparous females, oviparous females, and males. The production of oviparous females and males by the same mother is probably of quite common occurrence, but the dimorphic reproduction, including both agamic and sexual forms, appears to be rare. In the vast majority of cases one mother will produce only viviparous females or the sexes. It is of interest to note that in most of the cases recorded the agamic young were produced first and the sexes were the last forms produced by the mother.

#### OVERLAPPING GENERATIONS

Since the writers did not select the first and last young from each mother, they did not obtain exact data on the duration of each generation. However, using the average length of life of the various generations in

conjunction with their observations they can very closely approximate the true conditions.

In the diagram (fig. 2) the solid lines represent actual records. The hatched lines occurring at the beginning of the fifteenth to nineteenth generations are theoretical. They are necessitated by the fact that the earliest progeny was lost in some of these generations and it was necessary to continue with later offspring. The hatched lines at the end of the several generations are deduced by adding the average length of life to the date of last production of young.

It will be noted that theoretically all the generations from and including the seventh should be expected to produce the sexes. It is quite probable that such production occurs in nature, and that the sole reason sexes



FIG. 2.—Diagram showing the overlapping generations of the green apple aphid.

were not obtained from the seventh, eighth, and ninth generations is that later members of these generations were not reared and bred from.

#### FEEDING HABITS

As noted previously, the stem mothers fed only on the exposed green of the bursting buds and tiny leaflets, as this was the only food available to them. Later generations preferred the leaf petioles and then the young, newly formed twigs, although some remained on the leaves. In cases where the latter were excessively downy, however, the young stages especially appeared to find some difficulty in living on them. This character of downiness seemed to be particularly unfavorable when occurring on the underside of the leaves. Later, when the twigs commenced to harden, the aphides migrated back to the underside of the leaves, and in the fall, at the time the sexes began to appear, practically no viviparous aphides were found in any other location on the trees. This selection of food occurred only when the numbers were comparatively small. In the case of excessive infestation, twigs, leaf petioles, and the underside of the leaves are attacked simultaneously. Occasionally a single aphid will be found feeding on the upper surface of a leaf, but these cases are so rare as to be almost negligible.

In the writers' experiments the feeding of this species caused very little leafcurl. In the field, however, it often induces considerable curling, and some writers have recorded the injury as being very severe. This injury appears to be produced mainly by the earlier generations. The writers have had under observation some old trees whose water sprouts were heavily infested from the middle of the summer to the close of the season. Very few of the leaves on these suckers showed any curling and these few were only slightly affected, being merely rolled over somewhat. Certainly the curling produced by this species (Pl. LXXV, fig. 1) is never as severe as that caused by *A. malifoliae*.

It is very interesting, in this connection, to note that in the spring we seldom found large, pure colonies of *A. pomi* occurring on the trees. In practically every instance there were some individuals of *A. malifoliae* present. Since a single half-grown stem mother of the latter species can cause very severe curling it seems probable that many of the records of this effect from the feeding of *A. pomi* should properly be referred to the rosy apple aphid.

This species has been reported as attacking and injuring young fruit in some cases, and in severe infestations young aphides are often found clustered on the apples. A few experiments were performed along these lines, but the insects could not be induced to feed on the fruit in any instance, even when all foliage was removed from the twig. It seems very probable, therefore, that such feeding is rather rare.

The quality of the food has a very marked effect upon the size, color, and rapidity of growth of the insect (Table III). When furnished with tender succulent food throughout larval life, the adults are large, plump, and light green in color. On the other hand, if the food is poor in quality, the adults will be smaller, dark green, and the bodies will be much wrinkled. The insect will also require a considerably longer period to attain maturity on poor food.

TABLE III.—Effect of food on rapidity of development and reproduction of *Aphis pomi*

Poor food, insects small.				Good food, insects large.			
Experiment No.	Date born.	Nymphal period.	Number of young produced.	Experiment No.	Date born.	Nymphal period.	Number of young produced.
		Days.				Days.	
1559.....	Aug. 5	10	15	1617.....	Aug. 13	7	.....
1643.....	Aug. 14	10 to 12	8	1687.....	Aug. 19	7 to 8	28
1645.....	do.....	10 to 12	14	1839.....	Sept. 1	7 to 8	20
1488.....	July 28	11 to 13	44	1754.....	Aug. 21	7 to 8	25
1660.....	Aug. 17	12+	.....	1807.....	Aug. 27	7 to 8	25
1852.....	Sept. 10	12 to 14	.....	1856.....	Sept. 2	8 to 9	23
Average.....		11.5	10.25	.....		7.7	24.2



It will be noted that in general the smaller forms occurred earlier in the year than the large ones, at a time when the average length of the nymphal period was particularly short; also that, while the percentage of young produced by the larger insects is below the seasonal average, it is, on the whole, higher than the average of the period in which the insects occurred.

It is very difficult to judge exactly the condition of the food supplied to the insects. The size of the leaves furnishes no criterion as to the amount of food available. The aphides do as well on young, newly opening leaves as on larger ones. In fact the largest, plumpest aphides reared were fed on such foliage, while the poorest conditioned insects were raised on old, dark leaves, whose general condition can perhaps best be described as "hard."

Some of the dormant trees used in the spring continued to live throughout the season. These furnished very satisfactory food at first. They put out slender twigs which never hardened and the leaves of which never fully unfolded. During the latter part of the summer, while the foliage continued perfectly green and appeared to be very succulent growth practically ceased. Aphides confined on these plants grew slowly and never attained the size or plump condition of the average adult.

## SEXES

### OVIPAROUS FEMALE (PL. LXVII, FIG. 4)

#### DESCRIPTION

**FIRST INSTAR.**—Morphological characters: Antennæ as follows: I, 0.025 mm.; II, 0.032 mm.; III, 0.096 to 0.128 mm.; IV, base 0.042 to 0.056 mm., unguis 0.088 to 0.12 mm.; segments I and II with stout spinelike hairs, III and IV imbricated and bearing similar spines; segment III with a distal sensorium, and IV with the usual sensory group. Compound eye with about 14 facets. Labium about as long as the antennæ. Legs hairy, hind tibiæ about 0.209 mm. long.

Color characters: Very variable, usually an olive green, with dusky appendages.

**SECOND INSTAR.**—Morphological characters: Antennæ as follows: I, 0.028 to 0.042 mm.; II, 0.028 to 0.042 mm.; III, 0.06 to 0.112 mm.; IV, 0.048 to 0.08 mm.; V, base 0.058 to 0.08 mm., unguis 0.12 to 0.16 mm.; segment IV with a distal sensorium, and V with the usual sensory group, otherwise quite similar to antennæ of last instar. Compound eyes with about 24 facets. Labium nearly as long as III and IV of the antennæ. Cornicles thick, rounded at the tip. Legs more slender than in the previous instar; length of hind tibiæ, 0.256 to 0.32 mm.

**THIRD INSTAR.**—Morphological characters: Antennæ as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.16 to 0.176 mm.; IV, 0.109 mm.; V, base 0.08 mm., unguis 0.184 to 0.208 mm.; segments armed similarly to those of the previous instar. Compound eyes with many facets. Cornicles more cylindric than in the previous instars, 0.112 mm. long. Legs slender, hind tibiæ 0.112 mm. long.

Color characters: As in previous instars.

**FOURTH INSTAR.**—Morphological characters: Antennæ as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.096 to 0.16 mm.; IV, 0.08 to 0.152 mm.; V, 0.096 to 0.144 mm.;

VI, base 0.08 to 0.096 mm., unguis 0.192 to 0.256 mm.; segment V with a distal sensorium, segments III to V imbricated and with a few prominent spines. Compound eyes large and with very many facets. Cornicles cylindric, 0.161 mm. long, imbricated. Legs slender, hind tibiae 0.537 mm. long. Cauda conical, this and the anal plate densely setose.

Color characters: Approaching those of the adult, the dark green transverse band apparent in some cases, and the black portions more strongly developed than in the previous instar.

FIFTH INSTAR (ADULT).—Morphological characters: Antennae as follows: I, 0.064 mm.; II, 0.064 mm.; III, 0.176 to 0.192 mm.; IV, 0.112 to 0.16 mm.; V, 0.144 to 0.176 mm.; VI, base 0.096 mm., unguis 0.24 to 0.288 mm.; segments III to VI imbricated and with a few rather prominent spinelike hairs, without sensoria excepting the usual distal one on V, and the sensory group at base of unguis. Vertex very slightly rounded. Compound eyes large, with distinct ocular tubercles; prothoracic tubercle very large and distinct; abdominal tubercles small with the exception of the first cephalic pair and the pair caudad of the cornicles. Cornicles (Pl. LXXIV, fig. 14) subcylindric, tapering distad, imbricated and slightly flanged. Legs slender, and armed with stiff hairs. Hind tibiae slightly curved, very little, if at all, swollen, and armed with circular sensoria; these vary greatly in number, from a few to about fifteen (Pl. LXXIV, fig. 20). Three or four seem to be more common than the large numbers. They are very irregular in size, and are often very faint. Anal plate rounded, densely setose, and covered with a few long curved hairs on each side. Cauda somewhat elongate, conical setose, and armed with six or seven curved hairs on each side; length, 0.16 mm. Length of insect from vertex to tip of cauda, about 1.8 mm.

Color characters: Vertex and top of head dark brown to black. Thorax yellowish green, slightly pruinose. Anterior portion of the abdomen olive or greenish yellow, that portion just between and anterior to the cornicles dark green, forming quite a distinct band; segments of the abdomen caudad of the cornicles olive or yellowish green; margin of the abdomen with a row of dark markings. Cauda, anal plate, and cornicles black. Tarsi and distal extremities of tibiae, femora, and antennae dark brown.

In older specimens which have oviposited, the green band upon the abdomen becomes narrow and in very old specimens the body color often shows dark (dull) red-brown with the transverse band brighter than the remainder of the body. In a few cases the female is not olive or yellowish green as described, but is orange-yellow, of a color very similar to that of the males.

#### MALE (PL. LXVII, FIG. 2)

##### DESCRIPTION

FIRST INSTAR.—Morphological characters: Antennae as follows: I, 0.024 mm.; II, 0.032 mm.; III, 0.096 mm., IV, base 0.056 mm., unguis 0.088 mm.; segments I and II with a few stout bristle-like hairs; segments III and IV imbricated, the third one toward its distal extremity only and both with a few stout hairs; segment III with a distal sensorium, and IV with the usual group at the base of the unguis. Compound eye with 12 to 14 facets. Cornicles short, thick, and rounded at their distal extremities. Labrum about as long as segments III and IV of antenna. Legs thick and very hairy, hind tibiae 0.19 mm. long.

Color characters: Pale yellowish brown with dusky appendages and with the body often covered with a mealy bloom.

**SECOND INSTAR.**—Morphological characters: Antennæ as follows: I, 0.024 mm.; II, 0.032 mm.; III, 0.064 mm.; IV, 0.056 mm.; V, base 0.048 mm., unguis 0.096 mm.; segments with the characters of first instar, excepting that the distal sensorium is on segment IV. Compound eyes with about 18 facets. Cornicles short. Legs somewhat similar to those of the previous instar, hind tibiæ 0.192 mm. long.

Color characters: Similar to those of the previous instar. Tarsi, distal extremities of tibiæ, and distal extremities of antennæ black.

**THIRD INSTAR.**—Morphological characters: Antennæ as follows: I, 0.032 mm.; II, 0.04 mm.; III, 0.112 mm.; IV, 0.08 mm.; V, base 0.064 mm., unguis 0.112 mm. Armament of the antennæ, legs, etc., as in previous instar.

Color characters: As in previous instar.

**FOURTH INSTAR.**—Morphological characters: Antennæ as follows: I, 0.041 mm.; II, 0.041 mm.; III, 0.08 to 0.144 mm.; IV, 0.056 to 0.128 mm.; V, 0.072 to 0.112 mm.; VI, base 0.064 to 0.08 mm., unguis 0.128 to 0.176 mm.; segments III to VI imbricated and armed with a few stout hairs; segment V with a distal sensorium and VI with the usual group at base of unguis, otherwise the segments are similar to those of previous instar. Compound eyes with very many facets. Cornicles cylindric and imbricated, 0.072 to 0.096 mm. in length. Legs with many prominent spines, tarsi imbricated, tibiæ 0.368 to 0.448 mm. long.

Color characters: General color characters similar to those of third instar. Black marking only on the distal extremities of the antennæ, the distal extremity of the labium, the cornicles, the tarsi, and the distal extremities of the tibiæ.

**FIFTH INSTAR (ADULT).**—Morphological characters: Antennæ (Pl. LXXIV, fig. 9) as follows: I, 0.045 mm.; II, 0.045 mm.; III, 0.16 to 0.184 mm.; IV, 0.128 to 0.168 mm.; V, 0.112 to 0.144 mm.; VI, base 0.081 mm., unguis 0.184 to 0.232 mm.; segments III to VI strongly imbricated and armed with numerous stout hairs; segment III with 7 to 10 irregularly placed sensoria, the arrangement of these giving the segment a slightly knotty appearance; segment IV with about an equal number of sensoria irregularly arranged; segment V with about 5 sensoria of unequal size and with irregular arrangement; segment VI with the usual group at the base of the unguis. Vertex slightly rounded. Eyes with distinct ocular tubercles; thorax with a very prominent tubercle; abdomen with four lateral tubercles on each side, the pair caudad of the cornicles and the most cephalic pair larger than the others. Cornicles (Pl. LXXIV, fig. 13) cylindric, imbricated, slightly flanged distad, 0.104 to 0.28 mm. in length. Legs slender, hind tibiæ 0.496 to 0.592 mm. long. Cauda conical, not constricted, setose, and armed with long curved hairs. Anal plate somewhat truncate; genital plate rounded, wrinkled, and spiny; claspers irregular, corrugated, covered with minute spines; penis long, curved, fleshy (Pl. LXXIV, fig. 8). Length from vertex to tip of abdomen, about 1.12 mm. Shape of insect elongate and narrow, much more so than any other form.

Color characters: General color greenish brown, occasionally olive, sometimes with an orange tinge. Antennæ, cornicles, cauda, and genital appendages black; crown with a black cap similar to that of the stem mother; tip of the labium smoky to black. Insects sometimes slightly pruinose.

#### FIRST APPEARANCE OF SEXES

The production of the sexes is governed apparently by two factors, the season (temperature being of prime importance in this factor) and the generation. Of these the first is by far the more important.

The earliest sexes in breeding cages were born on September 2. They were in the eleventh generation, which was also the earliest generation in

which they occurred in the experiments.<sup>1</sup> Yet some viviparous insects of the sixteenth generation had been born as early as August 17, indicating very clearly that the season is of great importance in determining the production of sexual forms.

The evidence supporting the other factor is not quite so direct. The first sexes in the eleventh generation were born on September 2, in the twelfth and thirteenth, on September 5; in the fourteenth, on September 8; in the fifteenth, on September 22; in the sixteenth and seventeenth, on September 24. In all the generations up to and including the fifteenth, viviparous young were born on or before September 3.<sup>2</sup> In the sixteenth generation no young were produced between September 3 and 9, when viviparous young were born. The earliest vivipara in the seventeenth generation were produced on September 19.

The accompanying diagram (fig. 3) gives the curves for percentage of experiments containing sexes, by dates. Each date summarizes the production for seven days, the recorded date being the middle one of the seven. The writers can not give the exact percentage of sexes in each generation, since all of the progeny were not reared. However, of the generations occurring wholly after September 1, the sixteenth contained sexes in 51 per cent of the experiments, the seventeenth in 80 per cent, and the eighteenth in 100 per cent. In the nineteenth generation all the insects produced were oviparous females or males.

The most striking points brought out by these figures are that, besides the fact that each generation first occurs at a later period than its predecessor, an additional period is required (to and including the seventeenth generation) for the first appearance of sexes, and that in general the earlier generations are producing sexes in every experiment at a time when later generations are producing them in a very small percentage of experiments. This would indicate that, while seasonal climatic con-

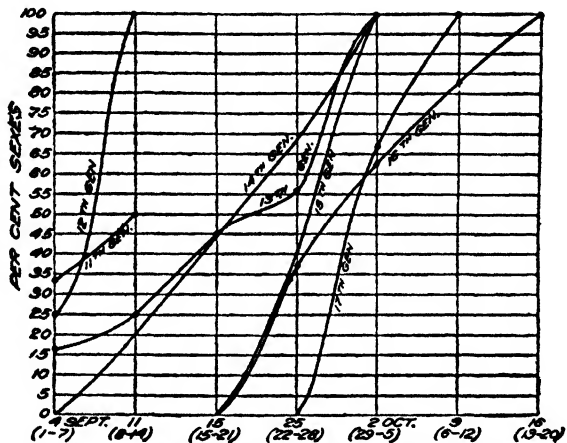


FIG. 3.—Diagram showing curves for percentage of experiments on the green apple aphid in which the sexes appeared.

<sup>1</sup> It seems probable that they may occur as early as the eighth generation under some conditions. See page 982.

<sup>2</sup> The insects born in the sixteenth generation before August 17 are not included in this discussion, since they failed to reach maturity, and it was necessary to go back two generations for new material.

ditions are the principal factor in the production of these forms, yet different, perhaps more severe, conditions are needed for each succeeding generation. Also, the generation itself becomes of more and more importance, till in the eighteenth (first produced on September 30) every experiment contains some sexes, while in earlier generations batches of young containing only parthenogenetic females were produced after that date. This latter point is emphasized by the fact that in the nineteenth generation only sexes appeared, while in the earlier generations some viviparous insects were produced as late as were any of the insects in the nineteenth.

It should be stated that the first sexes, in the open, were observed about September 15. These were partially grown. By September 22 adult and nearly full-grown males and females were abundant, indicating that these forms were produced at least as early as the 6th of September.

#### PERCENTAGE OF MALES TO FEMALES

Notes were not made in every case of the numbers of males and females in an experiment, but the records of 71 experiments in which such figures were kept give an average of 11 per cent of males in a total of 350 insects. This is above the true average, since many experiments contained "many females and no males," and such records have not been included. In only four experiments did the males outnumber the females, and in these experiments the greatest number of sexes raised was six.

#### LENGTH OF NYMPHAL LIFE

The period covered by the nymphal life of this form was considerably longer than that covered by the same stages of viviparous females, although there were only four nymphal stages, as in those forms. The average period for the immature stages was 20.6 days, the range being from 16 to 36 days. It was impossible to obtain satisfactory data as to the divisions of this period occupied by each stage, as in the majority of the oviparous females the normal rate of growth was considerably deranged by cold spells. Such conditions would greatly retard the development of the insect, with the result that the particular stage in which the insect passed through such temperatures was lengthened in comparison with the other stages. Thus, one experiment might show the first to be the longest stage, while in another the longest stage might be the third. In the case of oviparous females born early in September, the first three stages occupied about the same amount of time as the entire nymphal period of the viviparous females, while the last stage continued for about 6 days. Later in the fall it was impossible to make a comparison. The males require the same amount of time for complete development as do the females and the length of the nymphal period is affected by climatic conditions in exactly the same manner for both sexes.

## LONGEVITY

The longest record we have for total life of females is 47 days. At the end of this period the experiment containing two females was set aside and was not examined again for a month. At this time all were dead. The average life for the sexual females is about 25 days. The period varies with climatic conditions, insects born late in the season not living as long as those born in September. The total life period of the male appears to be considerably shorter than that of the female. The longest period observed was 31 days. In this case the male was never transferred from the plant on which it was born, and several females were present. When a male was transferred to a new tree bearing only one or two females, it usually disappeared within a week. In some cases it died, but often it could not be located at all. Toward the end of the season females were still quite abundant, but no males could be found.

The last oviparous females were observed, under natural conditions, on November 27. They were on a tree which still bore five or six green leaves. The next day these leaves fell and no more insects could be found. In the cages living oviparous females were present on January 5, at which time all experiments were closed.

## HARDINESS

This species, particularly the oviparous females, can withstand very severe temperatures. On January 5, 1915, observations were made on some experiments in the insectary. These experiments contained both viviparous and oviparous females. At this date all the viviparous and most of the oviparous females were dead. However, on one plant one living insect was found, while a second plant bore six insects which were alive. These latter six were very quiet, showing only the slightest movement when disturbed. The other one, however, was quite active and moved about on the plant. At the time the observations were made (2 p. m.) the temperature was 43° F., and these insects had been subjected to such low temperatures several times, the minimum being -6°.

## MATING

The oviparous females may mate within two days, and possibly in less time than that, after reaching maturity. On the other hand, a female may mate for the first time at least eight days after having become adult. The principal factor in determining this point is the facility with which the male finds the female.

Males have lived for considerable periods of time, as much as 10 days, and have spent much of the time on the same leaf with the female, and yet mating apparently did not take place. When males have been placed

beside females, even in contact with them, they have shown no signs of recognition. Sometimes they would remain by the female and commence feeding. Usually they would immediately wander away. Nevertheless, the male appears to be constantly searching for the female. Although it feeds considerably at periods, it is usually engaged in running rapidly about over the plant. The writers have seen such a male pass close to a female, which has produced one or more sterile eggs, several times and not pay the slightest attention to her. Some time later such a female would produce fertile eggs, proving conclusively that he finally found her. It may be that the female is only in condition to mate at certain times and that when not in condition she offers no attraction to the male.

The writers have never witnessed the entire act of copulation. A pair may remain in copula for at least 25 minutes, but whether or not the period is usually much longer than that is uncertain. During mating the female may move about carrying the male with her. She usually remains quiescent, however, with her beak inserted in the leaf or twig on which she rests.

Whether or not plural mating is necessary for fertilization of all the eggs is a point concerning which the writers are uncertain. It is indicated, however, by the fact that in a few cases females have laid fertile eggs and later sterile ones. Certainly plural mating takes place quite frequently. In one case under observation a female mated three times before laying any eggs, the first egg being produced between three and four days after the last mating observed. This is very difficult to explain unless the suggestion that the female mates only when in the proper condition is incorrect, in which case it is possible that the eggs were not fertilized by the first two matings. The writers have never observed females in copula after they have laid fertile eggs, but aphides which have laid sterile eggs frequently mate and produce fertile ones later.

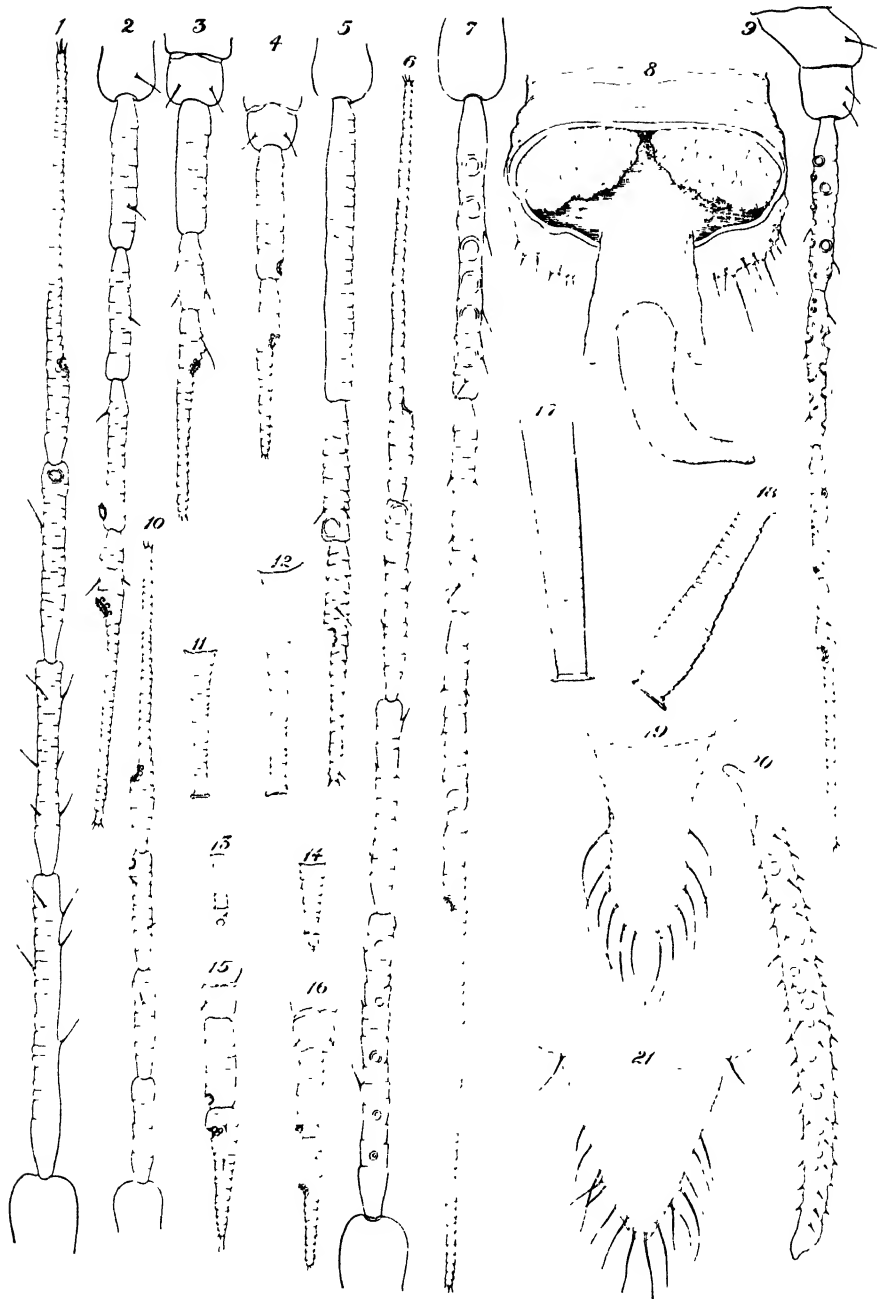
#### OVIPOSITION

The shortest time observed by the writers to elapse between mating and egg deposition is 2 days. However, in one experiment a female deposited a sterile egg on one day and a fertile one on the next. This would suggest very strongly that oviposition may take place within 24 hours after mating.

In the experiments the number of eggs laid by females ranged from 1 to 6. The normal number appears to be 6, though the average was 4.75. The rate of deposition is very irregular. In one case a female laid 2 in 24 hours and a third in the next 48 hours. In another case a female produced 3 eggs which were laid 6 and 5 days apart. In several cases females which had been observed in copula produced no eggs whatever, although living several days afterwards. On the other hand,







## PLATE LXXIV

Structural details of *Aphis pomi*, *A. avenae*, and *A. malifoliae*:

- Fig. 1.—*Aphis pomi*: Antenna of wingless viviparous female, adult.
- Fig. 2.—*A. pomi*: Antenna of wingless viviparous female, third instar.
- Fig. 3.—*A. pomi*: Antenna of wingless viviparous female, second instar.
- Fig. 4.—*A. pomi*: Antenna of wingless viviparous female, first instar.
- Fig. 5.—*A. pomi*: Antenna of stem mother.
- Fig. 6.—*A. pomi*: Antenna of intermediate.
- Fig. 7.—*A. pomi*: Antenna of winged viviparous female.
- Fig. 8.—*A. pomi*: Male genitalia.
- Fig. 9.—*A. pomi*: Antenna of male.
- Fig. 10.—*A. pomi*: Antenna of wingless viviparous female, fourth instar.
- Fig. 11.—*A. pomi*: Cornicle of winged viviparous female.
- Fig. 12.—*A. pomi*: Cornicle of wingless viviparous female.
- Fig. 13.—*A. pomi*: Cornicle of male.
- Fig. 14.—*A. pomi*: Cornicle of oviparous female.
- Fig. 15.—*A. avenae*: Antenna of stem mother, first instar.
- Fig. 16.—*A. pomi*: Antenna of stem mother, first instar.
- Fig. 17.—*A. malifoliae*: Cornicle of winged viviparous female.
- Fig. 18.—*A. avenae*: Cornicle of winged viviparous female.
- Fig. 19.—*A. pomi*: Cauda of adult.
- Fig. 20.—*A. pomi*: Hind tibia of oviparous female.
- Fig. 21.—*A. pomi*: Cauda of pupa.

PLATE LXXV

*Aphis pomi* on its host plant:

Fig. 1.—Colonies on apple.

Fig. 2.—Apple twig bearing eggs.





# SOILSTAIN, OR SCURF, OF THE SWEET POTATO<sup>1</sup>

By J. J. TAUBENHAUS,<sup>2</sup>

*Associate Plant Pathologist, Delaware Agricultural Experiment Station*

## INTRODUCTION

Soilstain of the sweet potato (*Ipomoea batatas*) is a disease which is little known. The present work is the result of three years' investigations by the writer.

The disease was first described by Halsted (3) in 1890 under the name "scurf." For the last 24 years nothing new has been added to our knowledge of this trouble; subsequent writers have merely quoted Halsted. From the writer's studies (8, 9) it became evident that the disease needed further elucidation. The average grower little suspects that "stain" is a fungus trouble. In fact, the term "soilstain" as applied by the grower indicates his belief that there is something in the soil which stains the roots. He even believes that the plant itself leaves some coloring matter in the soil which stains subsequent crops of this valuable root. Others think that the staining is due to the application of manure to the soil; hence, they term it "manure stain."

## ECONOMIC IMPORTANCE OF THE DISEASE

Soilstain is not a disease to be feared in the sense that it may produce a direct rot in the mature roots; nevertheless, it is economically important. Growers whose lands are badly infected assert that stained roots keep better in storage. Others find consolation in saying "there is no such thing as stain, the dark color of the skin being merely a varietal characteristic." The fact remains, however, that many eastern markets discriminate against stained roots. In years of overproduction the New York market refuses stained roots. The western buyers, on the contrary, are lax on this point; otherwise, many growers in the United States would be forced to cease producing sweet potatoes for want of a market.

## OCCURRENCE OF SOILSTAIN

Soilstain is prevalent in Delaware on practically all sweet-potato land. It has also been reported from other States where sweet potatoes are grown. The writer has met with it in the sweet-potato districts of Delaware, New Jersey, Maryland, and Virginia.

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<sup>1</sup> The Editorial Committee of the Journal of Agricultural Research kindly forwarded to the writer a copy of Harter's paper on "Sweet-Potato Scurf" before it was published, with the suggestion that reference to that article be made. The writer has covered certain studies on the scurf of the sweet potato in storage and has treated more fully the morphology and physiology of the fungus than has Harter. These studies verify the work of Harter with one exception; in the morphology of the fungus he overlooked the fact that the conidia are catenulate.

<sup>2</sup> The writer is indebted to Dr. Charles Thorn, of the Bureau of Chemistry, and Mrs. Flora W. Patterson, of the Bureau of Plant Industry, for having examined specimens of this fungus.

## SYMPTOMS OF SOILSTAIN

Soilstain is characterized at first by small, circular, deep-clay-colored spots on the surface of the sweet-potato root. These spots occur singly, but usually there are several in a given area. When very numerous, the spots coalesce, forming a large blotch which sometimes takes the form of a band or may cover the entire root. Soilstain is particularly conspicuous on the white-skinned varieties, such as the Southern Queen. Here the color of the spots is that of a deep-black clay loam. On the darker-skinned varieties the color of the spots does not appear so conspicuous. Soilstain is a disease of the underground parts of the plant. The vine and foliage are never attacked as long as they remain free from the soil. However, when these are covered, the petioles as well as the stems become infected.

## EFFECT OF THE DISEASE ON THE HOST

After several months of storage, badly affected roots become a deep brown, which greatly contrasts with noninfected sweet potatoes. Occasionally, badly stained roots seem to be subject to more rapid drying and shrinking. This, however, is not often the rule. Usually soilstain is very prevalent in overheated storage houses. It may be, therefore, that the rapid shrinkage is due to the overheating and not to the effect of the disease itself. More data are necessary to determine these points. Soilstain is not only a disease of the epidermis (Pl. LXXVII, fig. *a*) and as such considerably reduces the market value of mature roots, but it also attacks the very young rootlets, preventing their further development and indirectly reducing the yield. In badly affected fields the writer has estimated a loss of 10 per cent of the crop from rootlet infection.

## FACTORS FAVORABLE TO SOILSTAIN DEVELOPMENT

The type of soil seems to be a determining factor in the development of soilstain. Sweet potatoes grown on very light sandy soils, especially those which are hilly, are usually free from the disease. The heavier lands, or those rich in humus, rarely produce a clean crop. The application of manure favors the spread of the fungus and increases the stain. In fact, the manure itself is often a carrier of the disease, since diseased roots of all sorts find their way ultimately to the manure pile. The trouble is also carried directly with the seed stock. These, when planted in the seed bed, will produce 100 per cent of diseased sprouts. Experimental data, as well as extensive observations in seed beds and in the field, all corroborate these statements. Wet weather is favorable to the spread and increase of stain. During wet seasons the disease is more plentiful than in dry seasons.

## STORAGE EXPERIMENTS

Growers who do not suspect the fungous nature of soilstain are always at a loss to explain the appearance of the trouble in storage when otherwise healthy roots are brought in. In order to determine definitely the effect of storage on this disease, the following experiments were carried out during two consecutive seasons: At digging time in September, 1913, a diseased field was chosen for that purpose. A large number of roots were selected and placed in hampers in the following ways.

Experiment 1.—Three hampers were filled with roots which to all appearances were free from stain. The object of the experiment was to determine whether apparently clean roots taken from a diseased field will develop stain.

Experiment 2.—Three hampers were filled with roots which showed very slight infection. The spots in these cases varied from 5 to 10 in number and were single and scattered. The object of this experiment was to determine whether the disease would increase in storage and the spots coalesce.

Experiment 3.—Three hampers were filled with roots which were thoroughly stained all over. The object of this experiment was to determine whether badly affected roots would be subject to more rapid drying and shrinkage.

Experiment 4.—Three hampers were filled with well-stained roots. At the bottom was placed a layer of stained roots, followed by a layer of healthy ones, on top of which was another layer of stained roots. Each layer was separated from the other by a narrow strip of paper. The object of this experiment was to determine whether healthy roots in contact with diseased ones will become infected under storage conditions.

Experiment 5.—Three hampers were filled with roots which to all appearances were free from stain and were taken from an adjoining clean field. These were to serve as checks.

All the experimental hampers were placed in a medium-sized potato house which had poor facilities for ventilation. The conditions, therefore, were ideal for the experiment. The hampers were stored for a period of 5½ months.

The results of the above experiments may be summarized as follows: The roots in the first three hampers (experiment 1) remained clean, indicating that clean roots, though coming from an infected field, when stored and protected from contact with stained roots, will remain clean. The roots in the second three hampers (experiment 2) showed an increase in the stain and a coalescence of previously smaller spots. The roots in the third three hampers (experiment 3) seemed to be shrunken most. The roots in the fourth three hampers (experiment 4) indicated that apparently healthy potatoes may become stained when placed directly in contact with diseased roots. The check roots (experiment 5) were all free from stain. The above experiments were repeated in 1914 and 1915. The results obtained did not differ from those referred to above.



## CAUSE OF SOILSTAIN, OR SCURF

Halsted (3) was first to attribute the cause of soilstain (scurf) to a fungus, *Monilochaetes infuscans* E. and H. However, Halsted and the later writers have left no record of having experimentally proved the pathogenicity of the fungus. The writer has found no records of its having been grown in pure cultures. Several efforts by the writer to obtain the organism from badly stained roots which were kept in storage at first yielded negative results. Each time the causative fungus was overrun by a varied and rapidly growing flora. Pure cultures of the fungus were finally obtained from plantings of young minute spots. Of 300 such spots, 10 per cent yielded colonies of the causative organism, and these were few in number. The plates were examined every day and it was found that the fungus did not appear until nearly three weeks after culturing. Because of this slow growth, the fungus in previous work was overrun by secondary invaders. The cultural work emphasized the necessity of making a large number of poured plates when working with an apparently difficult organism. The first reference to the fact that this fungus had been grown in culture was made by the writer (8, 9) in 1914 and also recently by Harter (4). Using pure cultures of the fungus, the writer reproduced the disease several times at will.

## MORPHOLOGY AND PHYSIOLOGY OF THE FUNGUS

It has been stated that Halsted first named the organism. Although some figures are recorded in Halsted's bulletin (3), yet they are only fragmentary and do not take account of all the various stages of the morphology of the fungus. Halsted's observations of the fungus must have been limited to material on the host. In pure culture the fungus grows very slowly. It is characterized by small darkish round colonies (Pl. LXXVI, fig. 1) varying from one-tenth to one-fifth of an inch in diameter. The growth is floccose at the top, and anastomosed below, having a resemblance to a stroma in the substratum of the medium. The surface growth of a colony resembles that of species of *Alternaria* and some species of *Cladosporium*, but differing from these by its restricted slow growth. The surface of the colony of *M. infuscans* has an ashen color, which is also the general appearance of the fruiting. The fungus grows better on vegetable plugs and is at its best on steamed onion and celery stalks. The aerial mycelium is branched, septate, and hyaline when young (Pl. LXXVII, *n, w*). With age the mycelial cells turn gray, then black, and become filled with oil globules (Pl. LXXVII, *l, r*). The submerged hyphæ are made up of smaller cells which in old cultures swell and take on the appearance of chlamydospores. The conidiophores are distinct from the mycelium (Pl. LXXVII, *a*), and not obsolete, as stated by Stevens (7). From extended observations it was found that conidiophores do not arise in clusters, but are always formed singly

(Pl. LXXVII, *a, t, u*). They are erect, not branched, and when viewed hastily would be mistaken for setæ of species of *Colletotrichum* or *Vermicularia*. Upon a close examination they are found to be made of closely septate dark-celled mycelium, the base of which rests on one or two smaller ones (Pl. LXXVII, *a*). Generally the measurements of the conidiophores vary with the medium used. The host, too, seems to have a determining influence.

In material collected at random from the market or direct from storage the conidiophores appear to be smaller than those taken from artificially infected sweet potatoes. In the latter case, the causative organism seems to possess more vigor, because of moisture under control methods. The average of nearly 500 measurements on various media and on the host shows that the conidiophores vary from 100 to 300 $\mu$  in length. Great difficulty was experienced in studying the formation of conidia. It is difficult to observe spore formation on storage material. Harter (4) claims that there is but one conidium formed at one time at the tip of the conidiophore. As soon as this conidium breaks off, a new one is formed in its place. The studies of the writer on this point are at variance with those of Harter. The writer finds that the spores are borne in distinct chains. In pure culture the chains break up very readily when moistened and pressed down with a cover glass. The spore chains break immediately when moistened with alcohol, oil, or any other liquid (Pl. LXXVI, fig. 2, *k, d, b*). The chains of spores do not appear to be held together with any kind of mucilage. However, it was found that when a dry cover glass is carefully placed on the surface of a colony growing in a Petri dish and the latter placed under the microscope, all the stages of spore formation could be studied with much ease. The spores are borne in chains (Pl. LXXVI, fig. 2, *a, i*, and LXXVII, *g, h*). At first, the protoplasm of the tip of the conidiophore is seen to round up, then a minute bud pushes out (Pl. LXXVII, *c*) and increases in size until a mature spore is developed, which is left standing at the tip of the conidiophore (Pl. LXXVII, *d*). All the succeeding newly formed conidia are formed at the tip of the conidiophore, so that the oldest conidium stands at the farthest end of the chain (Pl. LXXVII, *e, f, i*). Careful observations of these chains have shown them to be made up of from 10 to 28 conidia. A distinct characteristic of the latter is that they are always guttulate (Pl. LXXVII, *m*), irrespective of the medium used. In some cases the conidia in pure culture appear to be massed in "pockets" around the tip of the conidiophore, as in species of *Gloeosporium* or *Fusarium* (Pl. LXXVI, fig. 2, *c, e, g, h, j*). However, a close examination will show that this is no definite characteristic of the fungus.

It has been stated that the least disturbance will cause the chains of conidia to break up. In so doing they invariably cluster around the conidiophore, grouping themselves in various ways (Pl. LXXVI, fig. 2,

*b, c, d, e, f, g, h*). This is observed only when the fruitings of the fungus are seen in a dry state. However, when placed in a drop of water or in any other liquid, the chains of spores break up and scatter over the liquid. The spores (conidia) are 1-celled, hyaline, with a greenish tinge, but never dark or brown. They measure from 15 to 20 by 4 to 6 $\mu$ . Sometimes a germ tube is produced at the tip of the conidiophore which later bears spores (Pl. LXXVII, fig. *h, j, k, o, p*). Broken-off mycelial cells are also capable of germinating. In this case a germ tube upon which spores are formed is first produced (Pl. LXXVII, fig. *b*). The spores readily germinate in water or in any nutrient medium (Pl. LXXVII, fig. *m, q, s, v, x, y, z*).

An attempt was made to determine whether *M. infuscans* would also cause a rot of the interior of the sweet-potato root. Inoculations made with pure cultures of the fungus in slits made with a sterilized and cooled scalpel showed the organism incapable of causing a rot of the root. It was thought that perhaps the starch or the sugar was detrimental, but the fungus grows well on a starchy medium prepared according to Smith (6, p. 196), although not so well on media rich in sugar. It seems probable that neither the sugar nor the starch restricts the growth of the organism to the epidermis only, but this is done by the enzymes of the host.

#### TAXONOMY OF THE FUNGUS

The name "*Monilochaetes infuscans*," meaning black bristly *Monilia*, given by Halsted to the soilstain fungus, remarkably describes the main features of the organism. However, Halsted failed to describe fully either the species or the genus. Saccardo (5) barely mentions the fungus. Neither Engler and Prantl (2) nor Clements (1) nor any other systematic writer on fungi record the genus *Monilochaetes*. The description given by Stevens (7, p. 597) is incomplete. It was probably taken from naturally infected material, where the chains of conidia are seldom, if ever, noticed, since they are partially broken off with the rubbed epidermis. The conidiophores in such material are often broken down or wanting. From the present studies it seems that the writer is warranted in retaining the names of both the genus and the species of *Monilochaetes* as used by Halsted. Harter (4), too, decided to retain this genus. The description from a pure culture follows.

#### *Monilochaetes infuscans* E. and H.

Spores borne in chains which readily break up; conidia hyaline to greenish, guttulate; conidiophores black, several septate; mycelium first hyaline, then darker with age. The submerged mycelium swells irregularly. Conidiophores, 100 to 300 by 3 to 7 $\mu$ ; conidia, 15 to 20 by 4 to 6 $\mu$ . The fungus is a very slow grower on artificial media. Parasitic on the sweet-potato root, causing a brown, blotched disease of the epidermis.

## SUMMARY

Soilstain, or scurf, is a disease of the epidermis of the sweet-potato root. The disease occurs in every sweet-potato section, East and South, and is probably generally distributed. It is more abundant in the heavier soils, especially where manure is used as a fertilizer.

Soilstain reduces the market value of the mature roots. It reduces the average yield by attacking also the younger rootlets and stunting their development.

Soilstain is a disease of the underground parts of the plant. In storage the disease spreads by contact and is favored by moist, poorly ventilated houses.

The fungus *Monilochaetes infuscans* is difficult to culture, because it is a very slow grower and is readily overrun by associated saprophytes. The conidiophores of *M. infuscans* are distinct from the mycelium, the older growth of which is also dark. The conidia are borne in chains which readily break up when moistened or disturbed.

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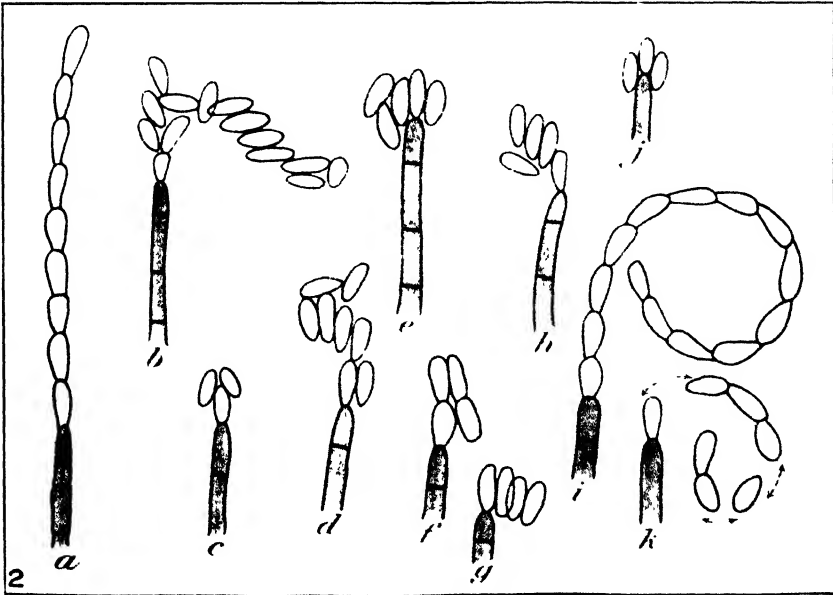
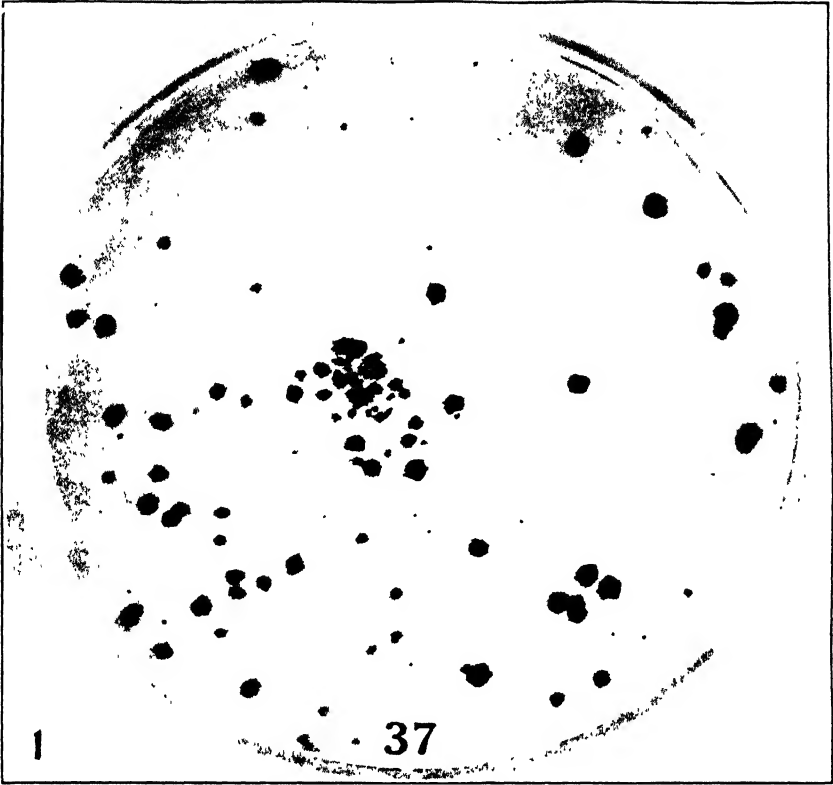
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PLATE LXXVI

Fig. 1.—Petri dish containing a pure culture of *Monilochaetes infuscans*.

Fig. 2.—*a*, Part of a conidiophore of *M. infuscans*, showing the unbroken chain of conidia; *b*, *d*, and *k*, various ways of the breaking up of the chains of conidia when disturbed or moistened; *c*, *e*, *f*, *g*, *h*, and *j*, spores collecting in pockets after the chains of conidia have broken up; *i*, bending in of the chain of conidia prior to breaking up into individual spores.

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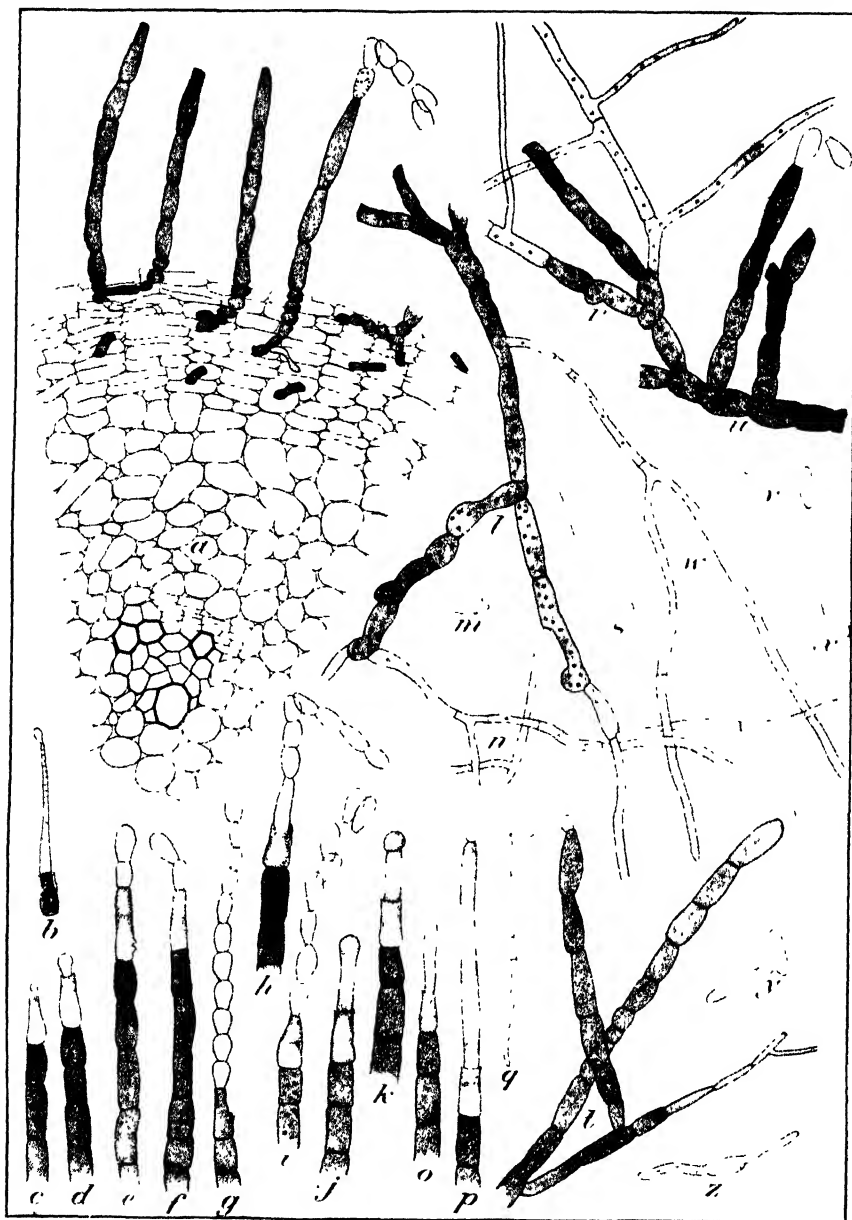


PLATE LXXVII

*a*, Part of a cross section of a sweet-potato root, showing the relationship of *Moni-  
lochaetes infuscans* to the epidermis of the host,

*b*, Germination of a fragment of mycelium of *M. infuscans*, showing the germ tube  
which is first produced and upon which conidia are borne,

*c*, *d*, *e*, *f*, *g*, *h*, *i*, and *t*, Different stages in the development of the spore and the  
chain of conidia;

*o*, *j*, *k*, and *p*, Protruding hyaline tube at the tip of the conidiophore on which are  
borne the conidia; this form of fruiting is not common,

*l*, *n*, and *w*, Differentiation of the coarser dark mycelium, and the finer hyaline to  
subhyaline hyphæ;

*u*, Attachment of the conidiophore to the mycelium;

*r*, Conidiophore-bearing mycelium, being part of *u*;

*m*, *q*, *s*, *v*, *x*, *y*, and *z*, Different stages in the germination of the conidia of *M.  
infuscans*.



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# JOURNAL OF AGRICULTURAL RESEARCH

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NO. 22

### AN ASIATIC SPECIES OF GYMNOSPORANGIUM ESTABLISHED IN OREGON <sup>1</sup>

By H. S. JACKSON,

*Chief in Botany, Agricultural Experiment Station of Purdue University, Indiana*

#### INTRODUCTION

Early in June, 1914, specimens of a species of *Roestelia* on Japanese pear leaves were sent to the writer from the office of the Secretary of the Oregon State Board of Horticulture. These had been collected in the yard of a Japanese family at Orient, in the vicinity of Portland, Oreg.

The writer visited the locality on June 11, 1914, and found two Japanese pear trees (*Pyrus sinensis*) the foliage of which was seriously affected with the fungus (Pl. LXXVIII, fig. 1). Since all species of *Roestelia*, so far as known, are the æcial stages of species of *Gymnosporangium*, and none are known to be perennial, it was at once recognized that the source of infection must be in the immediate vicinity. A search was made for a possible telial stage, but no positive evidence of the occurrence of such was obtained at that time, on account of the lateness of the season, though several varieties of *Juniperus*, as well as other members of the *Juniperaceae*, were found growing in the same yard, all of which were stated by the owners to have been directly imported from Japan several years before. Inquiry revealed that the rust had been present in small amount the previous season.

Careful examination showed that the rust should properly be referred to *Roestelia koreaensis* P. Henn., which was originally described from material collected in Korea (Chosen), but has since been reported as occurring commonly in Japan. An examination of the literature showed that considerable confusion has existed regarding the identity and relationship of certain of the Asiatic species of *Gymnosporangium*. Two species

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This paper is based on studies which were conducted in the laboratory of the Department of Botany and Plant Pathology of the Oregon Agricultural College Experiment Station. It is essentially as read at the summer meeting of the American Phytopathological Society, at Berkeley, Cal., on August 5, 1915, with certain additional information obtained from the examination of material in the herbarium of Dr. J. C. Arthur, to whom grateful acknowledgment is due for this privilege as well as for helpful suggestions.

See *abstracts in Phytopathology*, v. 5, no. 5, p. 293, 1915, and *Science*, n. s., v. 42, no. 1086, p. 582, 1915.

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have been especially confused, and on account of their interest in North America they will be discussed together in this paper. In order to make the situation clear, a review of the literature of these rusts with reference to their occurrence in Japan as well as in the United States will be given.

#### INVESTIGATIONS IN JAPAN

From 1897 to 1899 Shirai (7)<sup>1</sup> conducted infection experiments in which he claimed to show that *Roestelia koreaensis* was genetically connected with *Gymnosporangium japonicum* Sydow. He succeeded, in several different experiments, in obtaining the development of typical æcia of *R. koreaensis* on the leaves of *Pyrus sinensis* by exposing them to infection from germinating telia on *Juniperus chinensis*. Shirai stated, however, that in Japan the telia of *G. japonicum* occur not only on the trunks and branches, as the original diagnosis of Sydow states, but also on the leaves of the juniper, and he described and figured both stages (7, pl. 1, fig. 19 and 22).

Ito (4) recently called attention to the fact that Japanese mycologists have for some time considered that the forms which occur on the stem and leaves of *Juniperus chinensis* are not the same species. He also recorded the results of infection experiments in which the teliospores of the stem form were sown on *Pyrus sinensis*, *Amelanchier asiatica*, and *Pourthiaea villosa*, with infection only on the last. The resulting æcia proved to be typical of *Roestelia photiniae* P. Henn. Referring to the leaf form, Ito further stated that he considered it to be *G. Haraeanum* Syd. and that *G. asiaticum* Miyabe is synonymous. Miyabe and Yamada (6) have recently shown by infection experiments that *G. asiaticum*, which occurs on the leaves of *J. chinensis*, has for its æcial stage a species of *Roestelia* on *Pyrus sinensis*, *Cydonia vulgaris*, and *Cydonia japonica*. Hara (3) has also recently shown by infection experiments that *G. Haraeanum* has for its æcial stage *R. koreaensis* on *Pyrus sinensis*.

From the above it would appear that Shirai had both forms, *Gymnosporangium japonicum* and *G. Haraeanum*, mixed in the material which he used for inoculation and that his successful results on the pear were due to infection by the sporidia of the leaf form, *G. Haraeanum* (*G. asiaticum*), and not of the branch form, *G. japonicum*, as was supposed.

#### OCCURRENCE IN AMERICA

Clinton (1) reported the occurrence in 1911 of *Gymnosporangium japonicum* on imported plants of *Juniperus chinensis* in Connecticut. He also found the two forms on stems and leaves and followed Shirai in considering them identical. Long (5), after a study of Clinton's material, called attention to the difference between the two forms and described the leaf form as *G. chinense*, considering it distinct from *G.*

<sup>1</sup> Reference is made by number to "Literature cited," p. 1009.

*Haraeanum*. Clinton (2) later admitted that he confused two species, but believed Long not justified in describing the leaf form as new and considered *G. chinense* Long as synonymous with *G. Haraeanum*.

The branch form, *G. japonicum*, has recently (May 19, 1915) been collected on the campus of the University of Washington, at Seattle, Wash., by Dr. J. W. Hotson, and a specimen of it is in the herbarium of Dr. J. C. Arthur and has been examined by the writer.

#### OCCURRENCE IN OREGON

In the spring of 1915 (Mar. 29) the writer again visited the locality from which he had previously collected the material of *Roestelia koreaensis*. Within 20 feet of the two Japanese pear trees which had shown the infection the previous season and about midway between them two trees of *Juniperus chinensis* were found which showed abundant infection on the leaves of a telial stage of a species of *Gymnosporangium*. This was determined as *G. Haraeanum*. At the time the collection was made most of the sori had become swollen into gelatinous masses of characteristic shape (Pl. LXXVIII, fig. 3), though a few were found which had not become expanded (Pl. LXXVIII, fig. 2). No other species of *Gymnosporangium* was found in the vicinity, and no evidence of a branch form was noted.

A considerable quantity of this material was taken to the laboratory of the Department of Botany and Plant Pathology at the Oregon Agricultural College and used in greenhouse infection experiments. No plants of *Pourthiaea villosa* were available, but four potted plants of *Pyrus sinensis* and one each of *Pyrus communis* and *Cydonia vulgaris* were used in the experiments.

The method used was that of suspending branches of the infected juniper over the trees and covering them with large bell jars. This was done on March 30. These were left over the trees for four days, during which time the jars were removed for a few moments daily and the foliage and the inside of the jars sprayed with water. The plants were left covered longer than was intended, it having been the original plan to leave them covered only two days. At the time they were removed it was noted that evidence of infection was already visible on the foliage of the Japanese pear trees. Three or four days later it was evident that pycnia were developing in great abundance on the foliage of these and a few on the quince. There was evidence of initial infection on the trees of *Pyrus communis*, but no pycnia ever developed; only minute black spots finally resulted.

Fully developed æcia were collected from the infected trees of *Pyrus sinensis* (Pl. LXXIX, fig. 1) and *Cydonia vulgaris* (Pl. LXXIX, fig. 2) on June 3, though they were mature fully three weeks earlier. The resulting æcia were found to agree in all respects with the æcia collected in the field the previous year and with descriptions of *Roestelia koreaensis*.

These results, the writer believes, confirm the opinion regarding genetic relationships expressed by Ito and the culture work of Miyabe and Yamada and of Hara, referred to above. They also serve as additional evidence that Shirai's successful infections were obtained with the leaf form rather than with the branch form.

So far as the writer is aware, this is the first record of the complete establishment of any introduced species of *Gymnosporangium* in this country, though incomplete evidence of the establishment of the same species in California was brought to his attention through a specimen of *Roestelia koreaensis* found in the Arthur herbarium and collected on *Pyrus sinensis* at Oakland, Cal., July 1, 1913, and communicated by Prof. H. S. Fawcett, of the California Experiment Station. Correspondence with Prof. Fawcett and Prof. W. T. Horne, also of the California Experiment Station, revealed that the specimens came from a nursery conducted by Japanese, and that among other things various oriental evergreens were grown. The pears were said to have been originally imported from France in the dormant condition. The presence of this fungus on the leaves of the pears under the conditions is proof that the telial stage must have occurred on some species of *Juniperus* in the immediate vicinity, though no observations or collections were made. It is evident from this that the rust was at least temporarily established in California at that time.

#### TAXONOMIC CONSIDERATION

Based upon the results of the infection experiments discussed above, together with the evidence presented in the literature and such studies as the writer has been able to make with the material available in the Arthur herbarium, the present status of the species under discussion is believed to be as follows:

##### *Gymnosporangium koreaense* (P. Henn.), n. comb.

*Roestelia koreaensis* P. Henn., 1899, in Warburg, *Monsunia*, v. 1, p. 5.

*Tremella koreaensis* Arth., 1901, in *Proc. Ind. Acad. Sci.*, 1900, p. 136.

*Gymnosporangium asiaticum* Miyabe, 1903, in *Bot. Mag. [Tokyo]*, v. 17, no. 192, p. (34). (hyponym)

*Gymnosporangium Haraeanum* Syd., 1912, in *Ann. Mycol.*, v. 10, no. 4, p. 405.

*Gymnosporangium chinense* Long, 1914, in *Jour. Agr. Research*, v. 1, no. 4, p. 353.

Pycnia and æcia on Pomaceae: *Cydonia vulgaris* Pers., reported from Japan and cultured by Miyabe and Yamada; and from Oregon, cultured on June 3, 1915, by H. S. Jackson. *Cydonia japonica* Pers., reported from Japan and cultured by Miyabe and Yamada. No specimens seen. *Pyrus sinensis*, reported from Korea and Japan. (Part of type of *R. koreaensis*, examined.) Cultured in Japan by Shirai, Miyabe and Yamada, and by Hara. Occurred naturally at Orient, Oreg., on June 11, 1914 (H. S. Jackson), and at Oakland, Cal., on July 1, 1913 (H. S. Fawcett). Cultured at Corvallis, on Oreg., June 3, 1915, by H. S. Jackson.

Telia on Juniperaceae: *Juniperus chinensis*, reported from Japan (part of type of *G. Haraeanum*, examined) and from United States in a nursery at Westville, Conn., on stock just imported from Japan on March 28, 1911, by G. P. Clinton (type of *G. chinense*, examined), and from Orient, Oreg., on March 29, 1915, by H. S. Jackson.

*Gymnosporangium asiaticum* Miyabe is included here on the authority of Ito (4). Regarding *G. chinense*, the writer, after comparing portions of the original collection of this with a specimen of the type collection of *G. Haraeaeum*, is inclined to agree with Clinton (2) that they should not be separated. Long (5) gives us the most important basis for separating *G. chinense* from *G. Haraeaeum*, the presence of a single apical pore in the upper cells of the former species, found rarely in the thick-walled form, but more commonly in the thin-walled form. He states that in the latter there are two pores in the upper cells always occurring near the septum. A careful examination of a portion of the original collection of *G. chinense* in the Arthur herbarium shows that apical pores occur rarely, even in the thin-walled form, and in every case observed there was a second pore near the septum. The same condition was observed in the type material of *G. Haraeaeum*, though rarely. The collection of the writer, made in Oregon, also shows the same condition, but with the apical pores more abundant in the thick-walled form. In all of the collections examined spores were occasionally found in which one of the pores in the upper cell occurred at or near the septum and the other at a point from one-third to one-half the distance from base to apex. The other differences mentioned by Long are largely, the writer believes, due to variation and are not sufficient to justify separation.

***Gymnosporangium photiniae*** (P. Henn.) Kern, 1911, in *Bul. N. Y. Bot. Gard.*, v. 7, no. 26, p. 443.

*Roestelia photiniae* P. Henn., 1894, in *Hedwigia*, Bd. 33, Heft 4, p. 231.

*Gymnosporangium japonicum* Syd., 1899, in *Hedwigia*, Beibl., Bd. 38, No. 3, p. (141).

Pycnia and aecia on *Pomaceae*: *Pourthiaea villosa* reported from Japan, cultured successfully by Ito.

Telia on *Juniperaceae*: *Juniperus chinensis*, reported from Japan and from United States in a nursery at Westville, Conn., on stock just imported from Japan, March 28, 1911, by G. P. Clinton, and at Seattle, Wash., May 19, 1915, by J. W. Hotson.

#### ECONOMIC IMPORTANCE

Little is known concerning the economic status of the species under discussion. It may be said, however, that any fungus introduced from a foreign land is an unknown quantity and should be treated with suspicion until its status has been established. Several of the American species of *Gymnosporangium* are already of considerable economic importance, notably *G. juniperi-virginianae* Schw. in the eastern United States and *G. Blasdaleanum* (D. and H.) Kern in the Pacific States.

*Gymnosporangium koreaeense* has been shown to have its aecial stage on the cultivated quince and the Japanese pear. While attempts to infect *Pyrus communis* were unsuccessful, it should be pointed out that only a single attempt was made and it is reasonable to expect that certain varieties of pears, particularly those derived directly or by hybrid-

zation from the oriental species, would be susceptible to infection. It is not known whether this species is capable of infecting the apple. No records of its occurrence on that host have come to our attention.

While the only telial host known for either species is the Oriental juniper, it should be noted that this species is a very variable form, of which many varieties are recognized, and is closely related to several American species of the Sabina group. It is not at all impossible that either of the rusts under discussion might find a congenial host among some of the American species of *Juniperus* and become firmly established in this way.

The infection experiments of the writer with *Gymnosporangium koreaense* have shown that it develops very vigorously on the quince. Since the species of *Gymnosporangium* which are known to infect the quince do not usually develop so vigorously on that host as on others, the vigorous growth of this species on the quince may be an indication that *G. koreaense* is rather cosmopolitan in its habits and in a new habitat finally may prove capable of infecting a wide range of pomaceous hosts.

Several of the forms of *Juniperus chinensis* are commonly planted for ornament in various parts of the country, and practically all of these are imported directly from Japan. Both *Gymnosporangium photiniae* and *G. koreaense* are apparently common in Japan and, as shown by the American records, are liable to be frequently introduced on the telial host. If infected trees should be planted in the immediate vicinity of pomaceous hosts capable of harboring the æcial stage, it is possible for either species to become established, as has occurred in Oregon. In the case of the outbreak of *G. koreaense* in the nursery at Oakland, Cal., it is probable that the junipers which were the source of infection for the rust on the pears have been sold and distributed, and the rust may already be established in one or more localities that have not yet come to the attention of plant pathologists.

In the case of *Gymnosporangium photiniae* it is uncertain whether the telial stage is perennial or biennial. Clinton (1) records that an infected tree planted in the greenhouse developed after two years a new sorus in a different part of the stem than the point of original infection. It is known that several other related species which cause fusiform enlargements of the stem are perennial and take more than one season for the development of the telia after infection. As in all species of *Gymnosporangium*, the infection of the telial host occurs in the summer, and the mature sori do not develop till the following spring or, in some species, until the second spring after infection. *G. koreaense*, so far as known, is an annual form, requiring a new infection of the telial host each year.

In the case of either species it would be difficult to detect the presence of infection during the summer or dormant season, making inspection at the port of entry difficult. To be certain that infected junipers were

not planted, it would be necessary to hold all imported plants in quarantine until the following spring at least, in order to detect the presence of *G. koreaense* and until the second spring for the detection of *G. photiniae*. All trees found diseased should be destroyed, and in case the rust becomes established in any locality it would be advisable to remove the telial host.

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PLATE LXXVIII

Fig. 1.—Æcial stage of *Gymnosporangium koreaense* on under surface of leaf of *Pyrus sinensis*. Field collection at Orient, Oreg. Natural size.

Fig. 2.—Telial stage of *G. koreaense* on young twigs of *Juniperus chinensis*. Sori not distended. Field collection at Orient, Oreg. Natural size.

Fig. 3.—Same as figure 2, with sori distended.  $\times 2$ .

(1010)

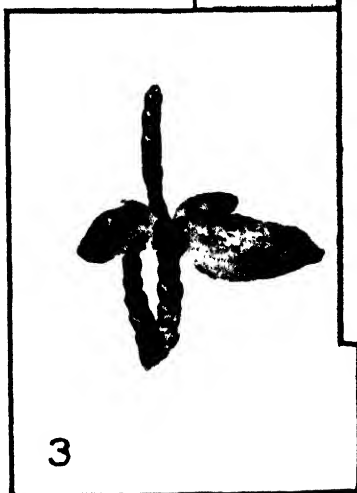




PLATE LXXIX

Fig. 1.—*Gymnosporangium koreaense* on leaves, petioles, and stems of *Pyrus sinensis*.  
The result of infection experiments using germinating telia on *Juniperus chinensis*.  
Natural size.

Fig. 2.—*G. koreaense* on *Cydonia vulgaris*. Natural size.



# RELATION OF STOMATAL MOVEMENT TO INFECTION BY *CERCOSPORA BETICOLA*<sup>1</sup>

By VENUS W. POOL, *Assistant Pathologist*, and M. B. MCKAY, *Scientific Assistant*,  
*Cotton and Truck Disease Investigations, Bureau of Plant Industry*

## INTRODUCTION

Leafspot infection of the sugar beet (*Beta vulgaris* L.) caused by *Cercospora beticola* Sacc. has been found to be closely related to if not directly controlled by stomatal movement in so far as the host is concerned. Penetration of the leaf by this parasite is effected, so far as known at present, only through open stomata. Consequently the factors favorable to stomatal pore opening become of fundamental importance in the occurrence of the disease.

The factors considered in this paper as most important in influencing stomatal movement are leaf maturity and certain environmental conditions. The term "leaf maturity" as employed in this paper is used to designate the condition of those leaves which have reached a maximum degree of physiological efficiency per unit area. Neither the size of the leaf nor its relative age in days can be taken as a reliable index to its degree of maturity. Under certain conditions young heart leaves of the sugar beet may be stimulated into physiological maturity before they have arrived at the average adult size, and such leaves will always remain small, while leaves which have attained average adult dimensions may still be physiologically immature. The varying degrees of leaf maturity have been found to be accurately indicated by the relative size and number of stomata per square millimeter of leaf surface, and these morphological factors have been observed to remain constant for a given maturity, even though the leaf size and position might indicate another stage of development. The stomata on leaves determined as mature by this method exhibited the greatest movement and responded most readily to changes in the environment. Light may be considered the essentially fundamental external factor affecting stomatal movement, although its influence may be greatly modified by different temperatures and relative humidities, the two factors that will be considered in detail in this paper.

In addition to stomatal movement, infection is also influenced by the rapidity of growth of the conidial germ tube and the maturity of the leaves. Detailed field observations have shown that heart and extremely

<sup>1</sup> This study has been carried on in connection with a detailed investigation of the sugar-beet leafspot conducted by the United States Department of Agriculture in cooperation with a beet-sugar company at Rocky Ford, Colo., during 1912 and 1913. A continuation of the entire problem was made possible during the season of 1914 at Madison, Wis., through the kindness of Dr. L. R. Jones, of the University of Wisconsin.

young leaves are not susceptible to infection, and that young mature leaves are only slightly so, while mature leaves show the greatest susceptibility. It has also been found that old leaves past their maximum development have for the most part lost their susceptibility, for they seldom show an increase in the number of leaf spots present. Thus the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, as they both occur on the leaves of the same degree of maturity.

With the varied host and environmental factors favorable, as might be indicated by the stomata on mature leaves remaining open for a period of from five to eight day hours and with vigorous viable conidia of the fungus present, infection would be practically assured.

### FACTORS INFLUENCING STOMATAL MOVEMENT

#### LEAF MATURITY

A study of the stomata on leaves of different maturities has indicated certain specific characters that might be used to determine the comparative development of different leaves. The number of stomata per square millimeter of leaf surface and the stomatal pore lengths have been found to give a good indication of leaf maturity as determined by the size, condition, and position of a leaf on a normal plant. By using the stomatal numbers and pore lengths as a means of measurement, the degree of maturity of any leaf on a heavily infected or otherwise abnormal plant may be determined, regardless of the degree of development indicated by its size and position. This becomes of especial value in the study of the leaves on a plant heavily infected by *Cercospora beticola*, for the young leaves may be mature, though their size and position would indicate immaturity.

Lloyd's<sup>1</sup> (7) method<sup>2</sup> for observing stomata in situ has been used throughout the study in determining the stomatal numbers and pore openings. Microscopic examinations were made near the middle of the blade of leaves which were taken directly from the plants to the stage of the microscope. Readings were continued not longer than two minutes, the stomata remaining unchanged during that time.

On a normally developed sugar-beet plant, pronounced differences are usually found to exist between the central, or heart, leaves, those occupying a midway position on the plant (here designated as mature leaves) and those occurring at the extreme outer portions of the leaf growth (old leaves). On leaves growing in such relative positions read-

<sup>1</sup> Reference is made by number to "Literature cited," p. 1038.

<sup>2</sup> Lloyd's stomatoscope (shown in Pl. LXXX, fig. 1), which was devised later, was kindly lent by the inventor for the studies which were made in Colorado in 1913. Two characters of this instrument, which make it exceedingly valuable for leaf study, are the long stage and the modified condenser, which serves also as a cooling chamber. The instrument also has a basal screw for tripod attachment. In a letter to the authors he has suggested (1) that the objective should be corrected for use without a cover glass, (2) that the focus of the condenser should be capable of being placed 5 mm. above the stage level for proper use in the case of thick leaves, and (3) that smoked glasses should be provided to shield the eyes.

ings were made of the stomatal numbers and pore lengths, together with the leaf size. These readings were taken during the same period and under comparable environmental conditions and the results are given in Tables I, II, and III, each leaf having been given the same number in all the tables.

## STOMATAL NUMBERS

It is shown in the general averages of Table I that the number of stomata per square millimeter of heart-leaf surface (289.8, upper surface; 353.5, lower surface) is more than  $2\frac{1}{2}$  times that on mature leaves (100.7, upper; 130.6, lower), as would be expected. There are in turn more on the mature than on the old leaves (80.1 and 105), while cotyledons have the fewest of all (54.7 and 73.2). The plants studied were grown in the field at Madison, Wis., under favorable conditions, and at the time the readings were made they appeared normal in every way. The older plants were about 7 weeks old, and those from which the cotyledons were studied were 3 weeks old. The cotyledons were green and turgid, comparing in maturity and activity probably with those leaves termed "mature." It may also be noted in the averages that more stomata were present on the lower surface of the leaves than on the upper and that the ratio between the two remained uniform.

TABLE I.—Average number of stomata on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings<sup>1</sup> taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average

Leaf No	Heart leaves		Mature leaves		Old leaves.		Cotyledons.	
	Upper	Lower.	Upper	Lower	Upper	Lower	Upper	Lower.
2.....			92.9 (3)	141.1 (2)	.....	.....	69.7 (4)	54.7 (3)
3.....	240.7 <sup>2</sup> (2)	282.2 (3)	94.6 (4)	124.5 (4)	53.1 (4)	78.0 (4)	66.4 (4)	92.9 (5)
4.....	293.8 (4)	325.0 (3)	94.6 (7)	126.1 (5)	59.7 (6)	102.9 (4)	53.1 (4)	78.0 (4)
5.....	275.5 (3)	391.7 (3)	104.5 (3)	132.8 (2)	71.3 (3)	86.3 (5)	59.7 (3)	59.7 (3)
6.....	298.8 (3)	373.5 (2)	124.5 (5)	129.4 (5)	74.7 (2)	99.6 (3)	76.3 (3)	.....
7.....	353.5 (3)	370.1 (3)	92.9 (3)	99.6 (3)	94.6 (4)	116.2 (3)	66.4 (4)	109.5 (3)
8.....	298.8 (1)	381.8 (2)	104.5 (3)	126.1 (3)	83.0 (2)	104.5 (3)	74.7 (2)	107.9 (4)
9.....	315.4 (1)	381.8 (1)	104.5 (3)	141.1 (2)	89.6 (4)	104.5 (3)	33.2 (3)	38.1 (3)
10.....	307.1 (2)	348.6 (2)	99.6 (3)	121.1 (3)	92.9 (3)	126.1 (3)	49.8 (3)	76.3 (3)
11.....	320.3 (3)	370.1 (3)	109.5 (3)	137.7 (3)	99.6 (3)	132.8 (3)	49.8 (3)	91.3 (4)
12.....	253.9 (3)	308.7 (3)	104.5 (3)	154.3 (3)	83.0 (1)	99.6 (1)	38.1 (3)	43.1 (3)
13.....							49.8 (4)	56.4 (5)
14.....	303.7 (3)	398.4 (1)	102.9 (4)	127.8 (4)	.....	.....	58.1 (2)	126.1 (3)
15.....			99.6 (2)		.....	.....	49.8 (4)	38.1 (3)
16.....			99.6 (2)	116.2 (1)	.....	.....	.....	.....
17.....	249.0 (1)	323.7 (2)	91.3 (2)	149.4 (1)	.....	.....	66.4 (3)	83.0 (1)
19.....			91.3 (2)	132.8 (2)	.....	.....	41.5	.....
20.....					.....	.....	33.2 (2)	49.8 (2)
21.....	257.3 (2)	340.3 (2)	.....		.....	.....	49.8 (1)	66.4 (1)
Average.	289.8	353.5	100.7	130.6	80.1	105.0	54.7	73.2

<sup>1</sup> These leaves were used for the readings given in Tables II, III, and V, and each leaf has the same number in all the tables.

<sup>2</sup> Numbers in italics indicate the maximum and minimum variation.



## STOMATAL PORE LENGTHS

The stomatal pore lengths of the different types of leaves show variations that are comparable to those observed in stomatal numbers—i. e., a smaller stomatal size must accompany the greater stomatal numbers per unit area. The pore lengths (Table II) of the stomata on the heart leaves ( $14\mu$ , upper surface;  $14\mu$ , lower surface) are on the average about half that of those on the mature leaves ( $28.5\mu$ , upper,  $27.1\mu$ , lower), and in turn the mature leaves show a slightly shorter pore length than those on the old leaves ( $31.06\mu$ , upper, and  $30.5\mu$ , lower) or cotyledons ( $31.8\mu$ , upper, and  $32.1\mu$ , lower), the last two sets being about equal.

TABLE II.—Average lengths (in microns) of stomatal pores on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings<sup>1</sup> taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average

Leaf No.	Heart leaves		Mature leaves		Old leaves		Cotyledons.	
	Upper.	Lower.	Upper	Lower.	Upper	Lower.	Upper.	Lower.
1.....			33.9 (2)					
2.....			33.9 (3)	27.5 (2)			32.1 (3)	36.6 (5)
3.....	12.2 (6)	10.5 (6)	29.6 (12)	26.2 (6)	33.9 (6)	33.9 (4)	33.0 (8)	28.3 (9)
4.....	12.7 (8)	14.0 (8)	25.8 (7)	26.7 (3)	30.9 (6)	29.6 (6)	33.9 (6)	32.5 (7)
5.....	11.8 (5)	13.1 (6)	28.3 (7)	25.4 (4)	29.6 (5)	29.6 (6)	29.6 (6)	39.8 (7)
6.....	18.6 (5)	15.6 (4)	32.5 (7)	26.2 (5)	31.3 (7)	30.0 (5)	40.2 (6)	
7.....	15.6 (7)	12.7 (5)	29.6 (9)	26.2 (4)	29.6 (3)	29.6 (4)	38.9 (5)	38.9 (8)
8.....	12.7 (5)	15.2 (5)	27.5 (7)	27.9 (6)			28.3 (7)	29.6 (6)
9.....	8.8 (7)	8.8 (6)	26.7 (3)	23.7 (6)			36.2 (3)	39.4 (3)
10.....	10.5 (4)	8.4 (4)	29.6 (6)	27.5 (4)			29.6 (4)	29.6 (5)
11.....	16.1 (5)	21.2 (4)	29.6 (12)	29.6 (5)			29.6 (5)	29.6 (4)
12.....	17.7 (5)	16.1 (5)	29.6 (4)	28.8 (6)			29.6 (4)	29.6 (4)
13.....							29.6 (6)	29.6 (8)
14.....	16.9 (4)	16.9 (3)	26.7 (6)	25.8 (7)			30.4 (5)	30.0 (6)
15.....			28.8 (6)				29.6 (6)	27.5 (6)
16.....			23.7 (6)	29.6 (5)				
17.....	14.8 (5)	14.8 (4)	27.5 (4)	27.5 (7)			29.6 (7)	29.6 (4)
18.....			27.5 (5)	27.5 (4)				
19.....			25.8 (9)	27.5 (9)			29.6 (12)	31.3 (9)
21.....			25.4 (3)	27.5 (4)				
Average..	14.0	14.0	28.5	27.1	31.06	30.5	31.8	32.1

<sup>1</sup> These leaves were used for the readings given in Tables I, III, and V, and each leaf has the same number in all the tables.

It thus appears that a definite relation exists between stomatal pore length and maturity of the leaf, although at times a shorter pore length might indicate the maturity as being somewhat less than would be shown by the number of stomata present. This may be due to the completed growth of the epidermal cells being attained before metabolic activity reaches its maximum, and consequently the stomatal pore length would be less.

## SIZE AND MATURITY OF LEAF

The sizes of the leaves from which the stomatal numbers and pore lengths have been taken show a difference that is characteristic of comparatively young plants during the early summer. As these plants increased in size, the oldest leaves would for a period be normally much smaller than the mature leaves, since the old leaves had been formed at a time when the plants were small. This difference in size is shown in Table III, where the mature leaves are much larger (18.3 by 15.1 cm.) than the old leaves (10.9 by 7.2 cm.), which in turn are only slightly larger than the heart leaves (9.9 by 6.6 cm.). Since the plants had not yet attained their maximum size, these heart leaves would, when mature, probably be larger even than the present mature leaves. Finally, however, a point would be reached where the mature leaves formed would not be increasingly larger with advanced age of the plants, at which time the mature and old leaves should be approximately the same size. It thus appears that there are great variations throughout the season in the sizes of the leaves that are developed at different periods or under abnormal conditions, owing to disease, unfavorable soil factors, etc. However, leaf maturity, regardless of leaf size, may be determined by the number of stomata per unit area and their pore lengths.

TABLE III.—Comparative sizes (in centimeters) of heart, mature, and old leaves and cotyledons of the sugar beet. Readings<sup>1</sup> taken at Madison, Wis., on July 6, 1914

Leaf No.	Heart leaves		Mature leaves		Old leaves		Cotyledons	
	Length	Width	Length	Width	Length	Width	Length	Width
1.....			18	17				
2.....			18	17				
3.....	10	6	11	16	10.5	7	2.5	0.7
4.....	14	9	21	16	8.5	7	2.3	.7
5.....	10	16	20	16	10	7.5	3.0	.8
6.....	10	5	20	16	17	10	2.0	.7
7.....	10	5	10.5	7.5	11	7	2.5	.7
8.....	8	6.5	20	16	8	4.5	3.0	.8
9.....	8	6.5	20	16	10	5.5	3.0	1.0
10.....	8	6.5	20	16	10	7	2.0	.6
11.....	10	3.5	20	16	12	8	2.5	.6
12.....	12.5	7	20	16	12	8	2.5	.8
14.....	8.5	4.5	20	16			2.4	.6
15.....							3.5	.8
16.....			18	15				
17.....	12.5	6	18	13			2.5	.8
18.....			18	15				
19.....			18	13			3.5	1.2
20.....							3.5	1.0
21.....	8	4					3.0	1.0
Average.	9.9	6.6	18.3	15.1	10.9	7.2	2.7	.8

<sup>1</sup> These leaves were used for the readings given in Tables I, II, and V, and each leaf has the same number in all the tables.

## COMPARISON OF FACTORS FOR DIFFERENT REGIONS

A comparison of the observations of stomatal numbers and pore lengths, leaf size and maturity at different times and places and under various conditions indicates the constancy of existing relations. These studies have been made in the field in Wisconsin and Colorado and in the department greenhouse at Washington, D. C. (Table IV). In general, the sizes of leaves are not comparable as read from these three places in that the periods of observation were varied and the controlling factors were different. However, the variations in the number and size of the stomata on the different leaves in a given locality have remained uniform in all readings.

The heart leaves, as would be expected, always exhibited more stomata per unit area and had shorter pore lengths than the mature leaves on the same plant, and, in turn, the mature leaves showed more stomata per unit area than the old mature leaves. It is to be noted, however, that heart leaves in Wisconsin, although comparing them with those studied in Colorado in stomatal pore lengths, showed twice as many stomata per unit area, indicating less maturity and consequently a greater possible ultimate development in area of leaf surface. This difference probably was due in great measure to the almost constant presence of leafspot on the plants observed in Colorado and the great freedom from it in the Wisconsin field from which the data were taken. The accumulative effect of the disease on the plant would be shown by the development of smaller sized leaves with a lessened number of stomata per unit area, showing that they were maturing at a size below normal.

TABLE IV.—Comparison of the average size of leaf, stomatal numbers, and pore lengths on different leaves of sugar-beet plants studied in Wisconsin, Colorado, and Washington, D. C.

Locality and leaf maturity.	Size of leaf		Number of stomata per square millimeter of leaf surface		Stomatal pore length.		Number of leaves in averages.
	Length.	Width	Upper	Lower	Upper.	Lower.	
Wisconsin: <sup>1</sup>	<i>Cm.</i>	<i>Cm.</i>			<i>μ</i>	<i>μ</i>	
Heart . . . . .	9.9	6.6	289.8	353.5	14.0	14.0	13
Mature . . . . .	18.3	15.1	100.7	130.6	28.5	27.1	16
Old mature . . . . .	10.9	7.2	80.1	105.0	31.1	30.5	10
Cotyledons . . . . .	2.7	.8	54.7	73.2	31.8	32.1	18
Colorado: <sup>2</sup>							
Old heart . . . . .	10.2	12.1	144.9	206.2	.....	.....	6
Old heart, uninfected <sup>3</sup> . . . . .	11.8	8.6	145.9	187.5	14.4	14.8	11
Young mature, infected <sup>3</sup> . . . . .	13.5	10.3	105.9	142.8	17.8	17.6	13
Mature . . . . .	16	14.4	80.4	109.6	19.4	18.1	26
Washington, D. C.: <sup>4</sup>							
Old heart . . . . .	5.3	3.1	161.0	.....	.....	.....	18
Mature . . . . .	6.7	4	98.0	.....	.....	.....	56
Old mature . . . . .	6.9	4.2	74.5	.....	.....	.....	57

<sup>1</sup> The results given are the averages taken from Tables I, II, and III.

<sup>2</sup> Readings made in the field from June to August, inclusive, 1913.

<sup>3</sup> The results given are the averages taken from Table X.

<sup>4</sup> Readings made during January, 1914, on potted plants about 8 weeks old grown in the greenhouse.

Mature leaves from Colorado have approximately the same number of stomata per unit area as old mature leaves from Wisconsin, although the stomatal pore lengths are less in the former than in the latter. This would seem to be due in part to the fact that the stomata read in Colorado were not open as widely as those read in Wisconsin, and thus their maximum pore length would not be attained when observed. However, the stomata which were well open in Colorado often had a pore length equal to the average in Wisconsin. The Wisconsin records include the readings made only early in the season on one day under favorable environmental conditions when the stomata were generally wide open. On the other hand, the Colorado records include readings made on various days throughout the season and often under unfavorable environmental conditions when the stomata were only slightly open, and thus they exhibited a short pore length. In such a case the stomatal numbers offer a safer criterion of leaf maturity than the stomatal pore lengths.

The number of stomata per unit area were also read on leaves from a normal mother beet plant growing in the field at Madison, Wis., on July 30, 1914, and the results obtained were entirely comparable to those from the first-year beets, in that leaf maturity could be indicated by the same stomatal numbers. The increase in number of stomata from the oldest, or basal, leaves to those occurring near the tips of the stalks, or the younger leaves, is shown in the following tabulation:

Length of leaf.	Width of leaf.	Average number of stomata per square millimeter of upper leaf surface.	Number of readings
<i>Cm.</i>	<i>Cm.</i>		
20	17	107.9	2
9	5	121.2	3
9	5	137.8	3
6	3.5	187.6	3
4.5	2	204.2	3
3	1.3	240.7	2

LEAF MATURITY AND STOMATAL MOVEMENT

Observations made at different times and on many plants have shown that the degree of stomatal movement is greatly influenced by leaf maturity. In the detailed tests reported, the readings of the stomatal pore widths on leaves of different maturities were made in the field at Madison, Wis., on a day when the sunlight was fairly strong and constant, the temperatures comparatively high, and the relative humidities above 60 per cent (fig. 1). This combination of factors was favorable for stomatal opening, as will be shown later under "Environmental factors." The leaves used in this test were the same as those from which the stomatal numbers and pore lengths have been given in Tables I, II, and III.

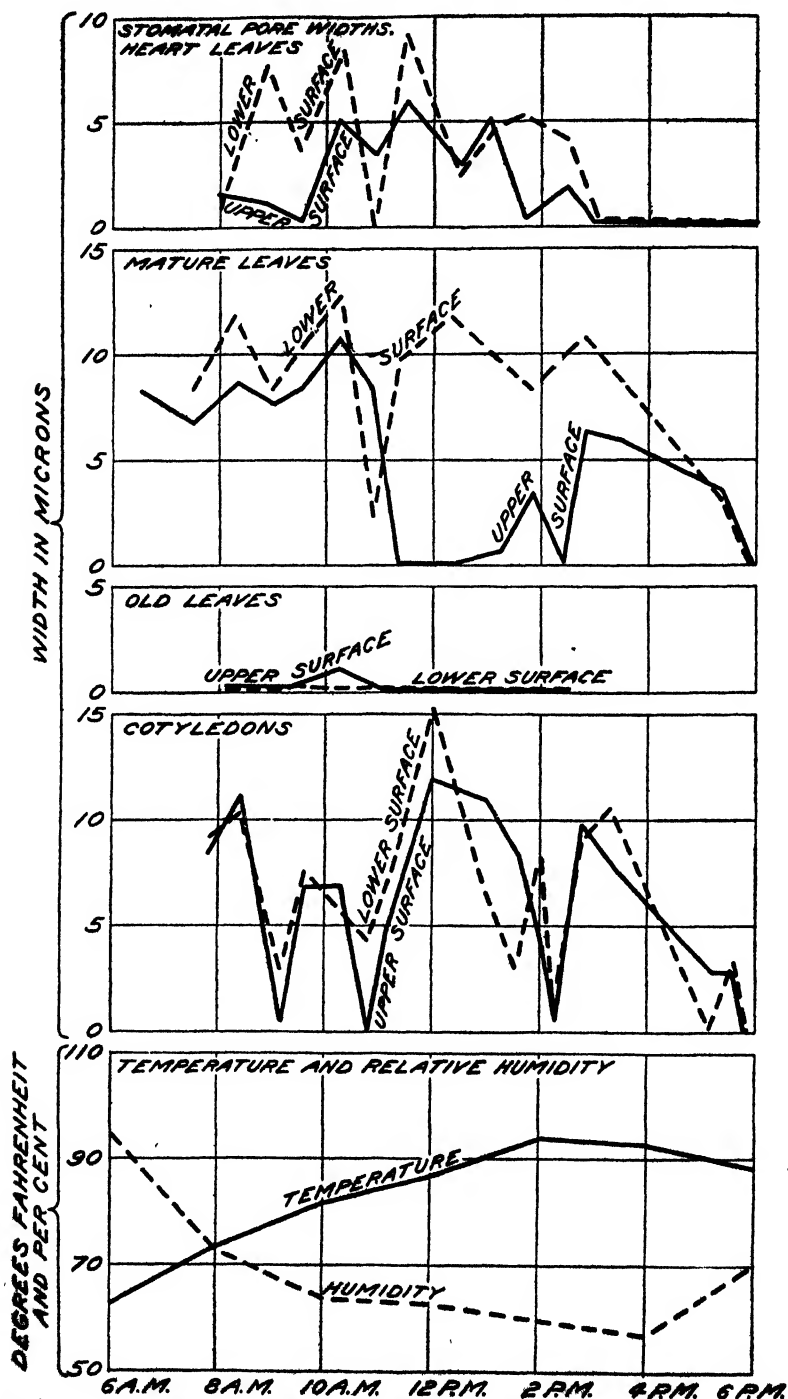


FIG. 2.—Stomatal pore widths on heart, mature, and old leaves and cotyledons of the sugar beet in the field, together with temperatures and relative humidities taken among the plants at Madison, Wis., on July 6, 1924 (Table V).

The results (Table V and fig. 1) show that the widths of the stomatal pores on cotyledons and mature leaves were greater than those on the heart leaves. In general, the stomata on the cotyledons and the lower surface of the mature leaves remained open throughout the day, while those on the heart leaves were entirely closed at 3 p. m. Those on the upper surface of the mature leaves showed a tendency to close from 11 a. m. to 1 p. m., and then to reopen before their final closure at 6 p. m. Shreve (8) found the stomata of *Parkinsonia microphylla* to exhibit this same tendency, since they closed partly during midday and reopened again during the afternoon. The stomata on the old leaves exhibited only slight movement and that on the upper leaf surface from 9 to 11 a. m. Readings were not made early enough in the day to determine the time of initial opening, but the curves indicate that the stomata on the heart leaves opened later than those on the mature leaves and cotyledons. This is shown in figure 1, in that at 8 a. m. the stomatal pore width on the heart leaves was very much less than on the mature leaves and cotyledons, being not more than  $2\mu$  on the heart leaves as compared to about  $9\mu$  on the others. On cotyledons the stomatal openings on the upper and the lower leaf surfaces remained quite comparable throughout the day. On the mature and heart leaves, however, the stomata of the lower surfaces exceeded in width of pores those of the upper surface. This relation was found to occur almost constantly throughout the day. In all cases the stomata on the upper surfaces closed at about the same time as those on the lower surfaces.

TABLE V.—Effect of leaf maturity on average stomatal pore widths on the upper and lower leaf surfaces of the sugar beet. Readings<sup>1</sup> were taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average

Leaf No.	Heart leaves.			Mature leaves.			Old leaves.			Cotyledons.			Time of reading.	Temperature.	Humidity.
	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.			
1	a. m.	μ	μ	a. m.	μ	μ	a. m.	μ	μ	a. m.	μ	μ	a. m.	°F.	
2	.....	.....	.....	6.30	8.4 (2)	.....	.....	.....	.....	7.50	8.4 (3)	9.2 (5)	6.00	61	95
3	8.00	2.5 (6)	0.3 (6)	7.30	6.7 (3)	8.4 (2)	8.05	0.4 (6)	0.0 (4)	8.25	11.3 (8)	10.1 (9)	8.00	74	73
4	8.50	2.1 (8)	7.6 (8)	8.15	7.6 (12)	11.8 (6)	8.50	4.6 (6)	0.0 (6)	9.10	4.6 (6)	2.9 (7)	.....	.....	.....
5	9.30	1.1 (5)	3.8 (6)	9.00	7.6 (7)	8.4 (3)	9.25	3.8 (5)	4.0 (6)	9.40	6.7 (6)	7.1 (7)	.....	.....	.....
6	10.15	5.0 (5)	8.4 (4)	9.35	8.4 (7)	10.5 (4)	10.10	1.3 (8)	0.0 (5)	10.20	6.7 (6)	.....	10.00	82	64
7	10.15	3.4 (7)	0.5 (5)	10.50	8.4 (9)	2.5 (4)	10.55	0.3 (5)	0.0 (4)	10.50	0.5 (5)	4.2 (8)	.....	.....	.....
8	11.30	5.9 (5)	9.2 (5)	11.20	0.7 (7)	9.7 (6)	11.25	0.5 (5)	0.0 (5)	11.15	5.0 (7)	7.1 (6)	.....	.....	.....
9	p. m.	.....	.....	p. m.	.....	.....	p. m.	.....	.....	p. m.	.....	.....	p. m.	.....	.....
10	12.30	2.9 (7)	2.5 (6)	12.15	0.3 (3)	11.8 (6)	12.25	0.6 (6)	0.0 (6)	12.00	12.7 (3)	15.2 (3)	12.00	87	63
11	1.05	5.0 (4)	4.6 (4)	1.10	8.6 (6)	9.7 (4)	1.20	0.4 (4)	0.0 (4)	1.00	10.9 (4)	6.7 (5)	.....	.....	.....
12	1.45	3.5 (7)	6.3 (4)	1.50	3.4 (12)	8.4 (5)	1.55	0.5 (5)	0.0 (5)	1.35	8.4 (5)	2.9 (4)	.....	.....	.....
13	2.25	1.7 (5)	4.2 (5)	2.20	0.5 (5)	9.7 (6)	2.25	0.6 (6)	0.0 (5)	2.00	4.6 (4)	8.4 (4)	2.00	94	60
14	2.55	0.5 (5)	2.3 (3)	2.50	6.3 (6)	2.6 (7)	.....	.....	.....	2.15	3.6 (6)	4.8 (8)	.....	.....	.....
15	.....	.....	.....	3.25	5.9 (6)	.....	.....	.....	.....	2.45	9.7 (5)	9.2 (6)	.....	.....	.....
16	5.30	0.5 (5)	0.5 (5)	3.25	5.2 (8)	4.6 (10)	.....	.....	.....	3.15	8.0 (6)	10.5 (6)	4.00	93	57
17	.....	.....	.....	5.25	3.4 (9)	6.3 (9)	.....	.....	.....	5.15	2.9 (7)	0.4 (4)	.....	.....	.....
18	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.40	2.4 (12)	3.1 (9)	.....	.....	.....
19	6.00	0.5 (5)	0.5 (5)	6.00	3 (3)	0.4 (4)	.....	.....	.....	5.50	0.5 (5)	0.6 (6)	6.00	88	70
	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.15	0.5 (5)	0.5 (5)	.....	.....	.....

<sup>1</sup> These leaves were used for the readings given in Tables I, II, and III, and each leaf has the same number in all the tables.

This, then, would indicate that the stomata on old leaves exhibit very little movement; that those on heart leaves open, but not so widely as on mature leaves, and close earlier; that on cotyledons and mature leaves they open widely, indicating their great activity. Therefore, in the study of the environmental factors influencing stomatal movement only mature leaves have been considered, since they were always available and responded readily to changes in environment. They also represent that portion of leaf growth which is most susceptible to infection by *Cercospora beticola*. If it is true, as claimed by Iljin (4) and others, that variation in the osmotic pressure of the guard cells regulates stomatal movement, then it might be concluded that the leaves which exhibit the greatest stomatal movement are also the most active metabolically and are consequently the most important to plant development.

#### ENVIRONMENTAL FACTORS

It is generally agreed by various investigators that the chief external factors influencing stomatal movement are light and temperature, while a difference of opinion exists as to the influence of relative humidity. Some believe that humidity greatly affects the degree of stomatal opening, while others consider it of only minor importance. Wilson and Greenman (12) found that the stomata on plants of *Melilotus alba* which were left covered with a glass case, thus being in a nearly saturated atmosphere, were well open, while on those which were left standing in the drier open air the stomata were nearly all closed. Darwin (2) gave evidence to prove that stomata were very sensitive to changes in the humidity, closing on being taken from a high to a low humidity and opening under the reverse conditions when all the plants were exposed to approximately the same light. According to Lloyd (6) "there is a small amount of evidence that a high relative humidity favors, as a condition, the wider opening of the stomata in the ocotillo" and in regard to *Mentha piperita*, also a desert plant, he concludes ". . . in these plants, that as long as wilting does not take place a low relative humidity does not reduce the stomatal opening."

As shown by the present study, the writers believe that, while light may be considered a fundamental factor in stomatal movement, yet stomatal closure is effected by low relative humidity, even though light is active. The relative humidity present at any time, together with an optimum temperature, has been found to be a good criterion of the amount of stomatal movement that may be possible under the existing conditions.

#### LIGHT

In this study no attempt has been made to determine the exact relation of light to stomatal movement. Only a few scattered readings have been made to determine what effect direct sunlight has on stomatal

opening (Table VI), and the results agree, in general, with those obtained by Lloyd (6) with desert plants. When the entire leaf was exposed to sunshine, as when the leaf blade stood parallel to the sun's rays, the stomata showed the same or a greater pore opening on the lower than on the upper leaf surface (series A). This was also found to be true with leaves entirely in the shade (series B). When the sun struck vertically upon the leaf blade, an accelerating effect on stomatal opening usually resulted, regardless of which surface was exposed to the sun (series C and D). This is also in agreement with the work of Balls (1, p. 231), in which he found that the stomata on the cotton plant opened widely in the sunlight and closed partly in the shade. The leaves in series C, read on July 18, indicate a point noticed by Lloyd (7) that the stomata near the apex of a leaf might have less pore width than those near the base, "a condition readily understandable if wilting is progressive from the apex of the leaf downward."

TABLE VI.—Effect of sunshine and shade on the width of the stomatal pore opening of the sugar-beet plant at Rocky Ford, Colo., in 1913

SERIES A (ENTIRE LEAF IN SUN)					
Date.	Hour.	Relative humidity.	Temperature.	Stomatal pore width.	
				Upper surface	Lower surface.
			° F.	μ	μ
May 17.....	7.15 a. m.	58	67	1.8 (3)	1.8 (4)
Aug. 4.....	7.30 a. m.	77	68	6 (6)	<sup>a</sup> 7.8 (7)
SERIES B (ENTIRE LEAF IN SHADE)					
May 17.....	7.15 a. m.	58	67	0 (6)	0 (6)
June 2.....	7.45 a. m.	100	65	1.5 (8)	<sup>b</sup> 6.3 (8)
June 3.....	9.30 a. m.	71	69	0	0
Aug. 4.....	7.30 a. m.	77	68	1.3 (7)	4.7 (5)
SERIES C (UPPER LEAF SURFACE IN SUN; LOWER IN SHADE)					
May 24.....	7.30 a. m.	69	68	4.2 (4)	8.6 (5)
May 26.....	1.45 p. m.	51	91	9.4 (6)	7.6 (4)
May 27.....	8.00 a. m.	62	78	4.5 (4)	0 (6)
July 18.....	9.00 a. m.	91	72	<sup>c</sup> 5.7 (3)	<sup>c</sup> 5.7 (3)
Aug. 4.....	7.30 a. m.	77	68	<sup>d</sup> 7.2 (3)	<sup>d</sup> 6.5 (3)
				5.7 (4)	0 (5)
SERIES D (UPPER LEAF SURFACE IN SHADE; LOWER IN SUN)					
May 19.....	8.00 a. m.	65	63	<sup>e</sup> 3.8 (7)	<sup>f</sup> 10.8 (6)
May 24.....	7.30 a. m.	69	68	2.5 (5)	3.4 (5)
May 26.....	1.45 p. m.	51	91	6.5 (6)	9.4 (5)
May 27.....	8.00 a. m.	62	78	1.0 (6)	7.4 (6)

<sup>a</sup> Wet from dew.  
<sup>b</sup> All wide open.

<sup>c</sup> Apex.  
<sup>d</sup> Base.

<sup>e</sup> Many closed.  
<sup>f</sup> All open.



## TEMPERATURE AND RELATIVE HUMIDITY

The determination of the effect of varied temperature and relative humidity on the opening of the stomatal pore of the sugar-beet plant was made under conditions which were somewhat under control. The plants used for study were first-year beets about 3 months old and of thrifty growth which had been grown in a deep soil bed in the greenhouse at Rocky Ford, Colo. A good root development was thus made possible, and normal leaf production had been accomplished. The leaves used for the readings were all mature and averaged about 14 cm. wide and 20 cm. long. Direct readings of the widths of the stomatal pores were made on plants both left free in the greenhouse and kept covered during the time of the experiment with a large glass humidity box (Pl. LXXX, fig. 2) of about 20 cubic feet capacity. This box was five-sided and could be placed over plants in a manner comparable to the bell-jar method. Aeration was made possible by this means and room was also available for a hygrothermograph, so that constant-humidity and temperature records were available without any disturbance of the plants. Comparable hygrothermograph records were also kept among the leaves freely exposed in the greenhouse and both instruments were checked by means of a cog psychrometer (Pl. LXXX, fig. 2). Middle-blade portions of different leaves were taken from all plants and stomatal readings made by the "in situ" method. The definite data of the experiments conducted on May 16, 17, and 20 and June 3 are given in Table VII and the graphic representations in figures 2 to 5.

TABLE VII.—Effect of varied temperature and relative humidity on stomatal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913. Comparable readings were taken in the greenhouse on plants covered by a large glass humidity box and on those left freely exposed to ordinary greenhouse conditions

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths. <sup>a</sup>		Temperature.	Relative humidity.	Average stomatal pore widths. <sup>a</sup>	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
<b>May 16.<sup>b</sup></b>	<sup>°</sup> F.	Per ct.	<sup>μ</sup>	<sup>μ</sup>	<sup>°</sup> F.	Per ct.	<sup>μ</sup>	<sup>μ</sup>
9.00 a. m. ....	68	70	9.0 (5)	7.9 (5)	77	43	1.8 (3)	0 (4)
1.30 p. m. ....	92	46	12.6 (4)	10 (4)	90	16	0 (5)	0 (5)
4.15 p. m. ....	89	54	8.6 (5)	0 (4)	93	18	0 (5)	0 (5)
7.00 p. m. ....	71	79	0 (5)	.36 (4)	75	24.5	0 (7)	0 (5)
<b>May 17.<sup>c</sup></b>								
5.00 a. m. ....	51	95	.36 (6)	2.1 (12)	52	73.5	0 (5)	0 (5)
7.15 a. m. ....	60	67	6.8 (6)	2.7 (4)	67	58	1.8 (3)	1.8 (5)
8.30 a. m. ....	63	66	7.3 (7)	8.2 (9)	71	50	2.5 (9)	2.1 (4)
10.00 a. m. ....	73	65	6.8 (4)	7.5 (4)	78	38	5.4 (4)	5.4 (5)
12.00 a. m. ....	80	63	7.2 (6)	9 (5)	83	31	1.8 (6)	5.4 (5)
1.30 p. m. ....	79	60	7.2 (4)	7.2 (4)	80	32	7.2 (3)	6.8 (4)
4.30 p. m. ....	70	74	7.2 (3)	6.4 (3)	71	34	0 (4)	0 (5)

<sup>a</sup> The number of readings is given in parentheses following each average.

<sup>b</sup> The sun shone brightly throughout the entire day.

<sup>c</sup> The sun shone brightly up to 4 p. m.

TABLE VII.—Effect of varied temperature and relative humidity on stomal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913—Continued

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths.		Temperature.	Relative humidity.	Average stomatal pore widths.	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
<b>May 20:</b>	<b>° F.</b>	<b>Per ct.</b>	<b>μ</b>	<b>μ</b>	<b>° F.</b>	<b>Per ct.</b>	<b>μ</b>	<b>μ</b>
5.00 a. m.	50	95	0.3 (6)	0.4 (13)	51.5	93	0 (7)	0.3 (9)
6.00 a. m.	51	95	.7 (8)	1.8 (9)	52	91	0.25 (11)	.2 (10)
7.00 a. m.	53	94	.3 (7)	1.6 (7)	54	83	.14 (10)	.28 (10)
8.00 a. m.	56	85	3.24 (7)	5 (6)	61	65	2.8 (6)	1.4 (8)
8.30 a. m.	63	76	2.16 (6)	2.8 (6)	63	64	2.1 (8)	2.1 (7)
9.00 a. m.	64	75	5.7 (6)	7.2 (5)	65	59	3.9 (6)	4.3 (6)
9.30 a. m.	64	75	6.1 (9)	7.2 (9)	65	59	4.6 (7)	3.8 (6)
10.30 a. m.	65	66	5.7 (6)	5.7 (4)	67	53	5 (6)	7.5 (7)
11.00 a. m.	68	65	7.2 (6)	7.2 (6)	68	57	1.8 (6)	1.4 (7)
11.45 a. m.	71	66	7.2 (4)	7.2 (4)	72	53	2.1 (6)	0 (5)
1.30 p. m.	75	57	9 (4)	11 (5)	74	45	1.08 (5)	1.08 (6)
2.15 p. m.	75	58	7.2 (6)	7.5 (5)	74	42	3.2 (7)	1.6 (6)
2.45 p. m.	73	57	7.2 (5)	7.2 (4)	71	42	3.6 (7)	4.06 (6)
3.30 p. m.	74	53	5.7 (5)	3.2 (5)	71	42	1.5 (5)	0 (5)
4.00 p. m.	75	53	6.8 (5)	6.1 (6)	74	40	2.1 (5)	0 (5)
<b>June 3:</b>								
7.45 a. m.	67	100	3.6 (3)	4.3 (4)	69	69	1.8 (4)	1.08 (4)
9.00 a. m.	70	100	6.3 (4)	7.38 (4)	67	64	2.5 (5)	.14 (5)
9.30 a. m.	72	100	7.5 (4)	7.2 (4)	69	71	2.8 (4)	0 (5)
10.00 a. m.	74	100	7.5 (4)	7.4 (4)	75	68	4.4 (5)	2.5 (5)
10.15 a. m.	80	100	7.2 (4)	7.3 (5)	73	75	6.1 (5)	6.1 (5)
10.30 a. m.	82	100	6.4 (4)	5.8 (4)	73	67	7.2 (4)	6.8 (5)
11.45 a. m.	93	100	9.3 (4)	9.3 (5)	79	62	4.5 (4)	9.1 (5)
12.15 a. m.	94	97	7.8 (4)	8.5 (4)	82	63	9.4 (3)	8.1 (3)
1.30 p. m.	96	93	7.02 (6)	7.5 (5)	80	56	3.8 (10)	2.7 (9)
2.00 p. m.	94	95	9.4 (6)	9.4 (6)	80	58.5	1.6 (11)	2.3 (12)
2.30 p. m.	85	95	7.8 (5)	7.8 (4)	75	57	5.8 (6)	0 (8)
3.00 p. m.	89	100	5.4 (6)	6.4 (6)	75	57	0 (5)	0 (5)
3.30 p. m.	75	100	5.4 (6)	4.3 (5)	75	57	0 (5)	0 (6)

<sup>a</sup> Intermittent clouds and sunshine up to 11.45 a. m., then bright sunshine until 2.25 p. m.; cloudy to 3.30 p. m., and then sunshine for the rest of the day.

Usually the temperature in the humidity box was practically the same as that outside in the greenhouse at the same time. Although no definite study has been made to determine the temperature most favorable to stomatal movement, it is to be noted that good stomatal opening occurred between 8 a. m. and 5 p. m., and during that time the temperature increased, on the average, from about 65° to 85° F. and decreased to 80° F. Only on June 3 was the temperature in the humidity box much higher than that outside in the greenhouse, and it appears that neither of these temperatures (96° in the humidity box and 80° in the greenhouse at 1.30 p. m.) produced a change in the degree of stomatal opening.

On the other hand, the humidity in the two places was quite different, being always higher inside than outside of the humidity box. To this difference in humidity the marked variation in the pore opening of the stomata has been attributed. For example, on May 16 the humidity ranged about 30 units higher inside than outside of the box (fig. 2), and the stomata were well open in the former place and closed practically throughout the day in the latter. On the upper leaf surface in the greenhouse only slight opening occurred at 9 a. m. and this disappeared

by 1.30 p. m. During this time the relative humidity fell from 43 to 16 while inside the humidity box it ranged from 70 to 46 and the average width of the pores of the stomata increased from 9 to  $12.6\mu$ . The stomata on the upper leaf surface were also open wider and remained open longer than those on the lower, while all were closed by 7 p. m. These points

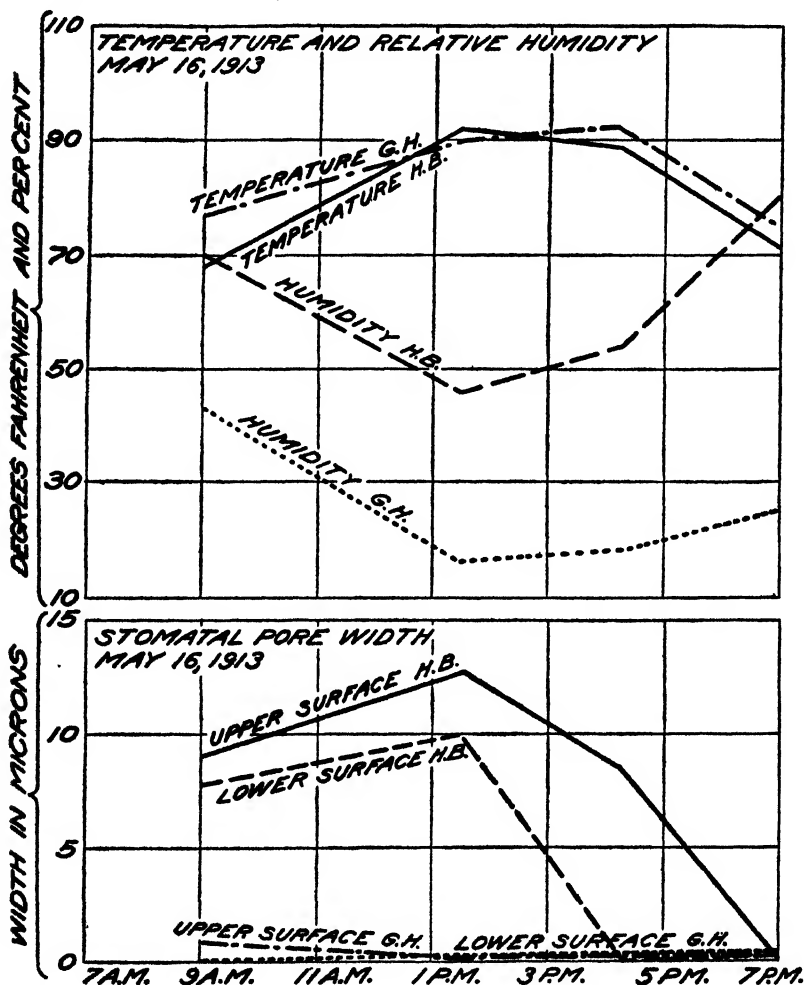


FIG. 2.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 16, 1913 (Table VII).

seem to indicate that the relative humidity as supported by soil moisture, transpiration, etc., must remain, in general, above a certain percentage in order that the maximum influence of light may be realized. Otherwise, if the humidity is too low, the light factor becomes in some way less operative, and the stomata open to a less extent and close earlier.

In another test made on the following day, the humidity ranged from 9 to 40 units higher inside the humidity box than in the greenhouse (fig. 3), and throughout the day the stomata were open wider in the former place than in the latter. At 5 a. m. all the stomata were closed except those on the lower leaf surface in the humidity box, which were slightly open. In general, the initial opening probably occurred soon after 5 a. m., for at 7.15 a. m. the stomata were all open, those in the humidity box being open wider than those outside. This point opposes the theory that the stomata in the humidity box remain well open during midday on account of the less intense light due to the additional window-

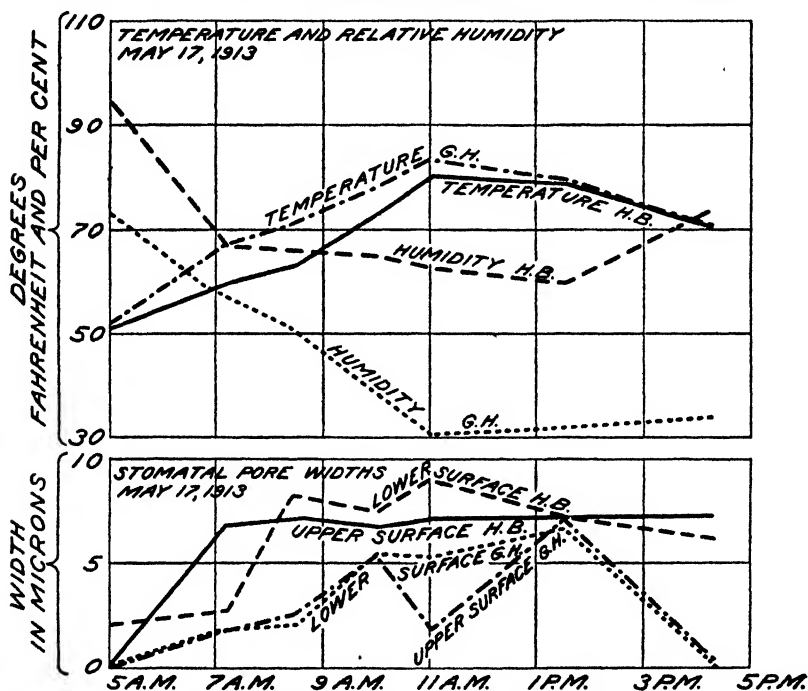


FIG. 3.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 17, 1913 (Table VII).

glass covering, while during the same period, those outside the humidity box close as a reaction to the more intense unobstructed light. If this were true, then, the stomata in the humidity box would open later in the day than those outside, because the light in the former place would be weaker. As a matter of fact, the stomata in the humidity box opened earlier and had greater pore width than those outside, even when thus exposed to the weaker light. The conclusion that may be drawn from this is that the relative humidity is the indicative factor of the causes which produce this difference. It should be noted that in the humidity box the humidity did not fall below 60 during the day, and the stomata were still open at 4.20 p. m., when the last reading for the day was made.

Outside in the greenhouse the humidity ranged from 31 to 34 after 11 a. m., and the stomata were entirely closed at 4.20 p. m.

A comparison of the stomatal pore widths of the leaves in the greenhouse on May 16 with those in the same place on May 17 shows that on the former day the stomata were practically closed all day, while on the latter they opened early and remained fairly well open till after 2 p. m. The humidity on the two days was quite different, being appreciably higher on the 17th than on the 16th. This offers an explanation for the differ-

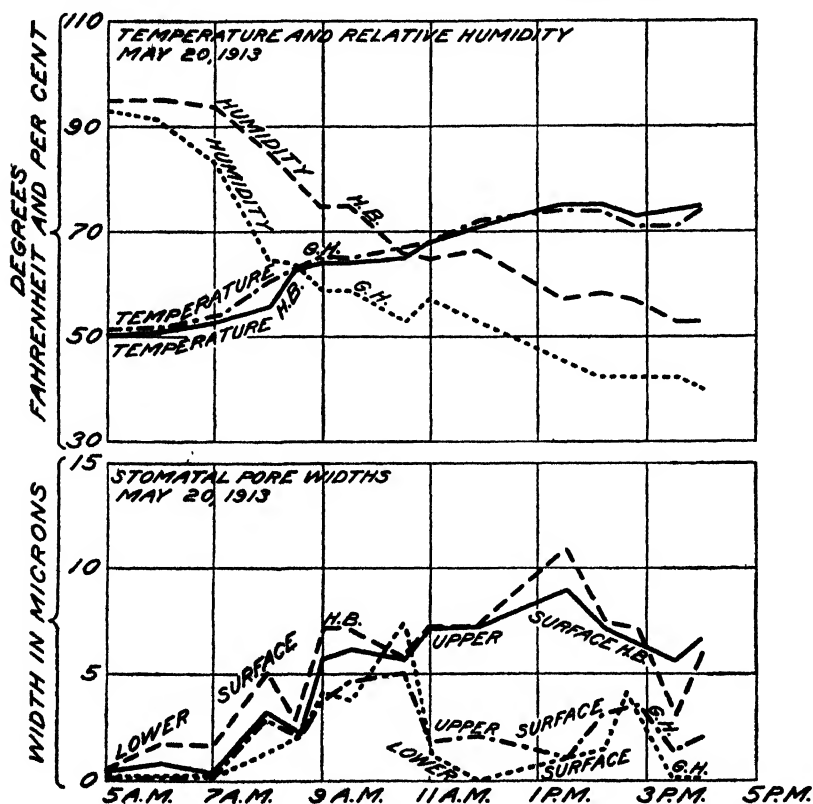


FIG. 4.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 20, 1913 (Table VII).

ence in stomatal pore opening, though, of course, conditions on the two separate days can not be compared too closely.

In another test, made on May 20, the stomata in the humidity box again showed greater widths of pores than those outside in the greenhouse (fig. 4) and the humidity ranged about 10 units higher throughout the day in the former place than in the latter. The greatest difference in the stomatal opening in the two places occurred after 11 a. m. when the stomata in the humidity box had much greater stomatal pore widths than those outside. The humidity remained generally near or above 60 in the box, while outside it was, on the average, below 50. The initial

opening in both places occurred about 5 a. m., and in the humidity box the opening on the lower leaf surface exceeded that on the upper, this relation remaining uniform throughout the day. This tendency is also indicated in figure 3 in the greater stomatal opening of the lower over the upper leaf surface in the humidity box. These observations in general agree with the findings of other investigators. Darwin (2) found that the stomata on the lower surface often opened earlier and remained open longer than those on the upper, though this was not always true. He believed that the difference in the opening was due to illumination rather than to any inherent distinction between the stomata. Livingston and Estabrook (5) found in the study of the stomata on several different

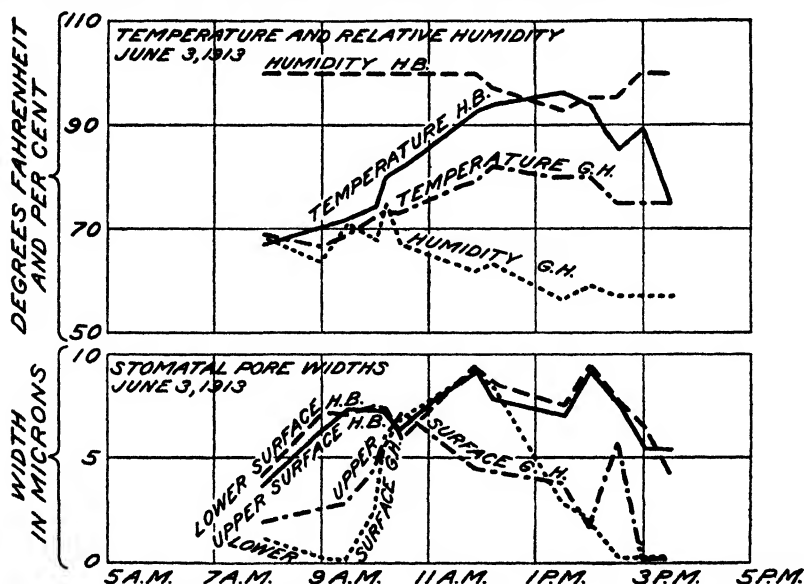


FIG. 5.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on June 3, 1913 (Table VII).

kinds of plants that those on the upper surface open and close more rapidly and close more completely than those on the lower. Lloyd (7) observed with cotton that—

The initial opening on September 30, 1911, occurred about 6.30 a. m., from which hour on a progressive opening movement was followed, the stomata of the lower surfaces opening somewhat in advance of those of the upper, though some exceptions to this appear.

Again, on June 3, after all the beds in the greenhouse had been watered on the preceding evening and the humidity box placed at that time over a portion of the plants for the test, the same general results were obtained, in that the stomata opened wider and remained open longer in the humidity box with higher humidity active for a longer period than in the greenhouse (fig. 5). During this test the stomata in the greenhouse remained open during midday till about 3 p. m., owing probably to the fact that

the humidity remained comparatively high—above 60. A comparable difference is noted in the humidities and stomatal pore widths taken on this date and on May 20. After 11 a. m. the humidity on June 3 was generally above 60 and the stomata had pore widths of more than 5 $\mu$  until after 1 p. m., when the opening gradually decreased until closure occurred about 3 p. m. On May 20, after 11 a. m., the humidity was generally slightly above 50 and the stomatal pore opening was reduced from 5 $\mu$  at 10 a. m. to about 2 $\mu$  at 11 a. m., after which time it seldom exceeded this amount.

A few readings were made in the field at various times during the season to get an indication of the stomatal movement under such conditions. On June 21 the stomata were found to be well open at 3 p. m. and later at a humidity of 60 or above (Table VIII). On June 23 the stomata were widely open from 8.30 to 10.40 a. m., even though the humidity dropped to as low as 40 at 10.10 a. m. The readings were not continued long enough to determine whether this low humidity would produce stomatal closure during midday. However, the readings taken on July 18 indicate that at 2 p. m. the stomata had a smaller pore width than at any other reading during the day and at that time the lowest humidity (57.5) of the day occurred. Two readings were made at the same time in this field. The one made near the center of the field, where the plants were large and close together, showed the stomata to be open (8.7 upper, 1.8 lower) at a humidity of 57.5, while the other made at the edge of the field, where the plants were small and far apart, showed the stomata to be closed at a humidity of 43.5. The maturity was determined to be the same for both sets of leaves used. In this case the soil-moisture content was noted to be much lower at the edge than in the center of the field, as the low humidity would indicate.

TABLE VIII.—*Stomatal pore openings on leaves of sugar-beet plants growing in the field at Rocky Ford, Colo., in 1913, together with the temperature and relative-humidity records taken among the leaves at that time*

Date and time of readings.	Temperature	Humidity.	Average stomatal pore widths. <sup>1</sup>	
			Upper leaf surface.	Lower leaf surface.
June 21:	° F.	85	10.4 (4)	10.08 (5)
3.00 p. m. ....				
3.45 p. m. ....				
4.30 p. m. ....	77	65	1.72 (9)	5.1 (7)
June 23:				
8.30 a. m. ....	74	60	10.8 (3)	9.9 (4)
9.20 a. m. ....	79	52	13.5 (4)	10.3 (4)
10.10 a. m. ....	83	39.5	10.6 (7)	7.1 (8)
10.40 a. m. ....	85	46.5	12.9 (5)	10.8 (3)
July 18:	72	91	6.3 (6)	6.4 (3)
9.00 a. m. ....				
10.30 a. m. ....				
11.15 a. m. ....				
2.00 p. m. <sup>2</sup> .....				
2.00 p. m. <sup>2</sup> .....				
2.00 p. m. <sup>2</sup> .....	89	43.5	0 (10)	0 (10)

<sup>1</sup> The number of readings made is given in parentheses following each average.

<sup>2</sup> These readings were taken at two different places in the same field.

Therefore, it may be concluded that if the relative humidity remains above 60 during the hours of daylight the stomata will probably be found open, while with a lower humidity the stomatal opening will decrease until it becomes greatly reduced and with still lower humidity the stomata may usually be found completely closed, or at least as nearly so as ever occurs. In an irrigated area especially, where the humidity is very largely controlled by the soil moisture, a high humidity may be directly due to a high soil-moisture content and would indicate increased plant activity. The beneficial effects of high humidity on increased plant growth is generally recognized. Wollny (13), who grew plants of barley, vetch, alfalfa, flax, and potato under conditions giving three degrees of humidity, found that with an increase in the degree of humidity there was an increase in the production both of the absolute quantity of fresh material and of dry matter. On the other hand, low soil-moisture content would greatly check such activities, and a low humidity, which would be associated with such a condition, would indicate marked differences in stomatal movement. Thus, it appears that a low humidity with its associated causes and effects results in diminished stomatal movement, and then the existing percentage of relative humidity becomes an important and convenient index to stomatal activities.

#### FACTORS INFLUENCING INFECTION

A consideration of the factors additional to, and somewhat preliminary to, stomatal movement that have been found to influence infection includes some of the conditions that affect both parasite and host in this relation. The effect that media, light, and temperature have on the rapidity of germ-tube growth becomes important in the relation that the fungus bears to leaf penetration. On the other hand, the maturity of the leaf, which controls stomatal mobility, plays a comparable part in this interrelation.

#### RAPIDITY OF GERM-TUBE GROWTH

No difference has been found to exist in the effect that north light and darkness have on the rapidity of germ-tube growth at a constant temperature. From the data given in Table IX it appears that all conidia germinated and had approximately the same average germ-tube lengths, together with a comparable average number of germinating cells per spore, regardless of the light factor. Consequently, under field conditions conidial germination would be expected to proceed equally fast under night or day conditions, except in direct sunlight, where the heat factor becomes important in causing rapid evaporation.



TABLE IX.—Effect of light and medium on the germination of conidia of *Cercospora beticola*, at a temperature of 24° C., on August 12, 1913, at Rocky Ford, Colo.

Environment.	Number of hours of growth.	Average percentage of germinating conidia.	Average number of cells per conidium.	Average number of germinating cells per conidium.	Average length of germinating tube.
Distilled water, north light.....	6¼	100	.....	2. 47	43. 28
Distilled water, dark room.....	6½	100	.....	2. 4	41. 11
Distilled water, north light.....	8	100	9. 42	4. 14	56. 31
Distilled water, dark room.....	8½	100	8. 69	3. 46	65. 77
Bean decoction, north light.....	9	100	9. 44	3. 33	55. 48
Irrigation water, north light.....	9¾	100	10. 16	3. 83	91. 69
Soil decoction, north light.....	10	100	6	3. 00	98. 42

Germination also occurred equally well in distilled water, bean decoction, soil decoction, and irrigation water, showing that a nutrient medium did not hasten germination nor did it retard it. It is also to be noted that the conidia were incubated nearly twice as long in soil decoction as in distilled water, which would account for the longer germ tubes in the soil decoction. In both solutions 100 per cent of the conidia germinated. The condensed moisture that may be found on leaves then would seem to give a favorable medium for conidial germination and that germ-tube growth could take place rapidly in it. It has been found that only a short time is necessary for germination to take place, since newly formed conidia may begin to germinate in three hours after being placed in water cultures at 26° C. The germinating tubes from such conidia may increase 5 $\mu$  in length in 40 minutes.

The effect of high temperatures on conidial germination is not considered in this discussion. However, in another phase<sup>1</sup> of the study of the sugar-beet leafspot, it has been determined that a period of days with extreme high night (70° F.) and day (104° F.) temperatures together with low relative humidity, a condition that may occur at times in an irrigated region, is inimical to the life of the conidia. This factor then becomes of importance in considering conidial growth and development under natural environment.

#### LEAF MATURITY

Near the middle of the summer or later, in a sugar-beet field infected generally with leafspot, the individual plant presents a typical picture of the disease. A cluster of uninfected heart and slightly infected young mature leaves occurs at the center of the plant, while all other leaves on the same plant are heavily infected. A comparison was made of the stomata on such heart and young mature leaves, or the oldest uninfected and the youngest infected leaves, on each of several plants. The study

<sup>1</sup> The thermal relations of the fungus will be discussed in a later paper entitled "Relation of climatic conditions to infection by *Cercospora beticola*."

was carried on in August, 1913, near Rocky Ford, Colo., and the readings of the two types of leaves from the same plant were made near together so that all time factors might, so far as possible, be eliminated. The results show that on the average the number of stomata is less and their pore length is greater (Table X) on the infected leaves than on the uninfected, showing the greater maturity of the former. Some variations in these numbers occur, but it is to be noted that the four infected leaves with the greatest number of spots present have, on the average, fewer stomata per square millimeter of leaf surface and a greater stomatal pore length than the four infected leaves with the least number of spots.

TABLE X.—Comparative average maturity of *Cercospora beticola* infected (young mature) and uninfected leaves (heart) of the sugar-beet plant as shown by the number and pore length of the stomata. Readings<sup>1</sup> taken on August 5 to 11, 1913, at Rocky Ford, Colo.

INFECTED YOUNG MATURE LEAVES<sup>2</sup>

Leaf No.	Size of leaf.		Average number of stomata.		Average stomatal pore lengths.		Number of leaf-spots per leaf.
	Length.	Width.	Upper leaf surface.	Lower leaf surface.	Upper leaf surface.	Lower leaf surface.	
	<i>Cm.</i>	<i>Cm.</i>					
1. ....	17. 5	12. 5	98. 4 (3)	123 (1)	19 <sup>μ</sup> (3)	19 <sup>μ</sup> (2)	24
2. ....	17	12. 5	68.06(3)	106. 6 (3)	19 (5)	19 (3)	21
3. ....	9. 5	9	95. 1 (3)	111. 5 (3)	19 (6)	19 (3)	21
4. ....	14. 5	10	102. 5 (4)	127. 9 (3)	19 (4)	19 (2)	14
5. ....	10	7. 5	106. 6 (3)	155. 8 (2)	19 (6)	17. 5 (5)	9
6. ....	10. 5	9	110. 7 (2)	139. 4 (2)	19 (5)	19 (5)	5
7. ....	15	10. 5	77. 9 (2)	123 (2)	15. 2 (6)	19 (6)	5
8. ....	11. 5	12	114. 8 (2)	137. 3 (4)	17. 1 (6)	15. 2 (2)	3
9. ....	15	12. 5	118. 9 (2)	164 (2)	15. 5 (5)	13. 3 (2)	3
10. ....	10. 5	8. 5	114. 8 (2)	172. 2 (2)	19 (4)	19. 7 (5)	1
11. ....	13. 5	9. 5	133. 9 (3)	183. 1 (3)	15. 2 (4)	16. 7 (7)	1
Average.	13. 1	10. 3	103. 8	140. 3	17. 8	17. 8	.....

UNINFECTED HEART LEAVES<sup>2</sup>

1. ....	14. 5	9. 5	123 (2)	164 (1)	19 (3)	17. 4 (3)	.....
2. ....	14	9. 5	118. 9 (2)	172. 2 (1)	15. 9 (5)	15. 2 (5)	.....
3. ....	8. 5	8	145. 2 (3)	147. 6 (3)	17. 1 (8)	15. 2 (5)	.....
4. ....	12. 5	8	135. 3 (2)	166. 4 (3)	13. 1 (4)	15. 2 (2)	.....
5. ....	13	8	133. 9 (3)	184. 5 (2)	13. 6 (6)	15. 2 (4)	.....
6. ....	10. 5	7	144. 3 (3)	174. 6 (3)	15. 2 (3)	17. 1 (2)	.....
7. ....	13	9. 5	131. 2 (1)	205 (2)	11. 4 (4)	13. 3 (6)	.....
8. ....	9	9. 5	127. 1 (2)	184. 4 (2)	13. 9 (5)	11. 4 (4)	.....
9. ....	13	10	192. 7 (2)	225 (2)	13. 3 (8)	13. 3 (6)	.....
10. ....	9	6. 5	192. 7 (2)	241. 9 (2)	13. 3 (4)	15. 2 (6)	.....
11. ....	12. 5	9. 5	161. 2 (3)	196. 8 (3)	12. 1 (7)	14. 4 (5)	.....
Average.	11. 8	8. 6	145. 9	187. 5	14. 4	14. 8	.....

<sup>1</sup> The number of readings made per leaf is given in parentheses following each average.

<sup>2</sup> Infected leaf 1 was on the same plant as uninfected leaf 1, infected leaf 2 was on the same plant as uninfected leaf 2, and so on through the series. The leaves of each pair were read at the same time.

The averages for the eight leaves mentioned are:

Leaf No.	Size of leaves.		Number of stomata.		Length of stomatal pores.		Number of leaf-spots.
	Length.	Width.	Upper.	Lower.	Upper.	Lower.	
1 to 4.....	<i>Cm.</i> 14.6	<i>Cm.</i> 11	91	117.2	<i>Cm.</i> 19	<i>Cm.</i> 19	20
8 to 11.....	12.6	10.6	120.6	164.1	16.7	16.2	2

It is also to be noted that infected leaf 11, which had only one spot, had the shortest average stomatal pore lengths (except leaf 9) and the highest number of stomata per area of any of the infected leaves studied. From these figures it would further appear that of all the uninfected leaves studied, only leaf 1 would have a stomatal count and pore length that would indicate leaf susceptibility. It might be concluded that this leaf remained uninfected merely by chance and that the others were uninfected because they had not as yet reached the maturity which would allow infection to occur.

Detailed field observations made of the amount of infection that appeared on the different leaves of many sugar-beet plants during an entire season have again shown that the greatest number of leafspots developed on the mature leaves. The records from one plant are shown in Table XI. The leaves were tagged and numbered consecutively, beginning with the outermost, or oldest, so that the new leaves tagged on all days after the first one were heart leaves. As these grew older they became susceptible to leafspot, and with increased maturity usually became heavily infected, and finally the death of the leaf occurred. Those leaves, whose numbers are in *italic*, on the last date reported were killed by the fungus. From 400 to 1,000 spots were sufficient to kill a leaf, depending on its size, in a few days. While the death of many of the leaves not reported as killed by *Cercospora beticola* was no doubt hastened by the presence of the fungus, yet age and other factors were predominating causes of the death of the leaf.

The results obtained show that, as a rule, infection did not take place readily on old yellow leaves, but occurred most readily on active green leaves. It is true that there was often a large increase in the number of spots present on the leaves during the few days just previous to the death of the leaf, as is shown by leaves 21, 24, 25, 27, 35, and others on this one plant (Table XI), but such leaves were not normally old. They were no doubt green and quite active when infection took place and merely died prematurely and very suddenly as a result of the great number of spots produced.



leaves were killed by the fungus, the plants were forced to produce more new leaves in an effort to keep up their normal activities. Under such conditions the new leaves formed, appeared to mature earlier than usual, and never became as large as normal. Thus, they became susceptible to infection by *Cercospora beticola* quite early in their development, and often became infected while comparatively small.

This difference in the susceptibility of the different leaves is shown in a general way in Table XI by the diagonal grouping of the three types of leaves—namely, the very young, the mature, and the old. The upper diagonal indicates either no increase in spots on the old leaves, or a slight increase on those which were still somewhat active. The lower diagonal indicates the very young leaves on which there occurred few or no spots, while the middle section represents the mature, active leaves of the plant on which the greatest increase in infections took place. A great increase in the number of infections developed on either the same leaf (reading to the right) or on the entire plant (reading diagonally) as the season advanced.

The mature leaves therefore show the greatest susceptibility to leafspot infection and possess the characters which allow the freest penetration of the host tissue by the fungus. Such leaves, as previously shown, have on the average a stomatal count on the upper surface of approximately 100 per square millimeter with a stomatal pore length of  $28\mu$  and exhibit the greatest stomatal movement. Thus, the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, for they both occur on the leaves of the same degree of maturity.

#### STOMATAL MOVEMENT AND GERM-TUBE PENETRATION

It may then be concluded that a favorable daily temperature ( $70^{\circ}$  to  $90^{\circ}$  F.), combined with a relative humidity which does not fall below 60 at any time, together with daylight, will offer conditions under which the stomata on the mature leaves should remain open throughout the day. This condition of the host associated with favorable growth factors for the parasite would usually allow germ-tube penetration and leafspot development.

With these factors active in producing stomatal opening, detailed studies were made of germ-tube penetration from material that had been collected in the field during controlled tests. For these experiments newly formed conidia from recently developed leaf spots were sprayed on mature sugar-beet leaves about 7 p. m. After an incubation period of 11 days numerous typical leaf spots appeared. Portions of these leaves were taken 24, 36, 48, 60, and 72 hours after inoculation, killed and stained according to modifications <sup>1</sup> of the method given by Vaughan

<sup>1</sup> These modifications were suggested by Miss Pearl M. Smith, of the Botany Department of the University of Wisconsin. After the acetic alcohol had acted for 22 to 24 hours, the material was washed for 6 to 8 hours in 95 per cent alcohol, stained in Planes's stain overnight, and destained with acid alcohol until the leaf tissue became a clear red, or even pink in places. The material was washed in 95 per cent alcohol until the acid was removed and mounts made in Euparal. Balsam, as a mounting medium in these studies, was not found to give a good differentiation between the stomata and the penetrating fungous mycelium.

(11). An examination of several hundred slides prepared at different times by this method from inoculated leaves has shown that conidia may germinate, produce long germinating tubes and yet not penetrate closed stomata (fig. 6). On the other hand, wherever penetration was found to occur, the stomata were open, and although it has long been known that this organism gains an entrance through the stomata, this point has never been mentioned. Thümen (9, p. 50-54) seems to have been

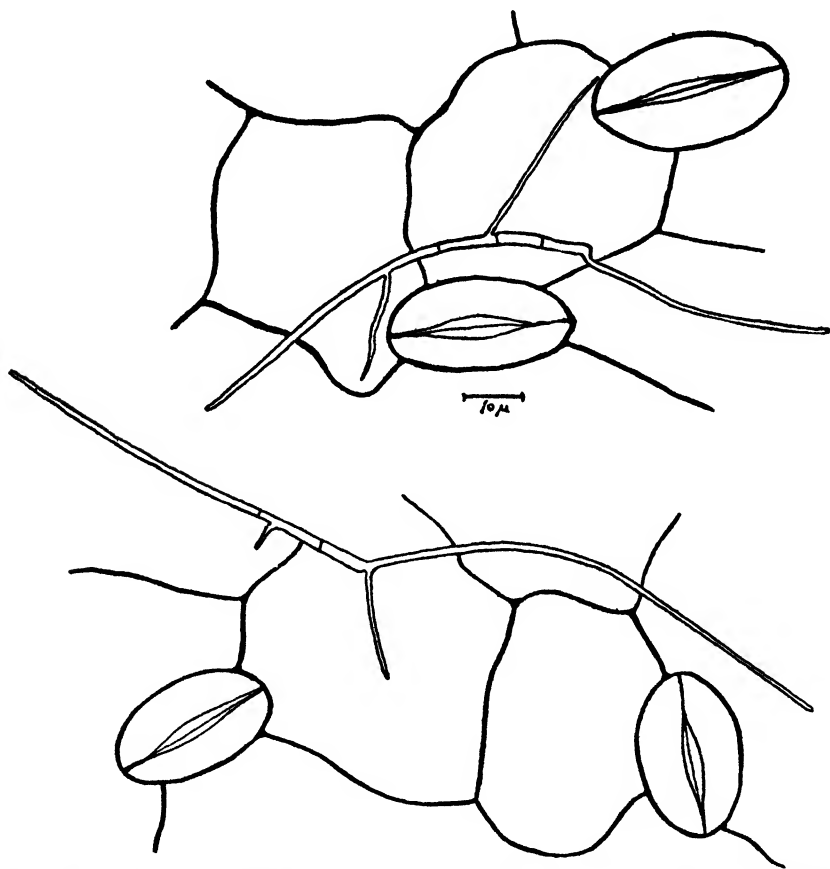


FIG. 6.—*Cercospora beticola*: Conidia germinating on a sugar-beet leaf, but germ tubes not entering or being greatly attracted by closed stomata.

the first to state that a spore which is carried by some means to a green and yet not too old, and thereby hardened, beet leaf, is able to germinate in the shortest time, penetrate into a stoma, and form a number of hyphæ. Fraenkel (3) also agrees with this observation, adding that it is characteristic that the tufts of conidiophores grow out of the stomata. However, no mention seems to be made of the stomatal movement necessary for host penetration.

As soon as penetration of the stoma was gained by the germ tube, a marked change was noted to take place in the character of the fungous growth produced, as indicated by different staining qualities. The conidium and the slender germ tube external to the spore opening stained lightly, while the cells in the pore opening or beneath the stoma stained much more deeply and were comparatively large and round (Pl. LXXXI, A, B, F). It was only rarely observed that penetration into two different stomata took place by germ tubes from one conidium (Pl. LXXXI, B, b). In the case observed, the two stomata were near each other and slight germ-tube growth was sufficient for the penetration of both. As a rule, however, only one germinating tube from a conidium has been found to penetrate the host tissue, although it is known that, if this tube does not penetrate before its desiccation takes place, another cell of the conidium may germinate later before the entire conidium loses its viability and penetration might again be possible. (At times the pore wall of a guard cell may be penetrated and the growth gradually spread to the adjoining epidermal cells (Pl. LXXXI, F, c). (Normally, however, the germ tubes grow through the pore opening, probably receiving some stimulus from the guard cells and form round, heavily staining mycelial cells which pile up directly in the air chamber below the pore opening. The fungus then grows toward the parenchyma cells (Pl. LXXXI, C, d) and flatten out against their walls, probably for nutritive purposes. At times, without further development within the host, the fungus grows back out through the stoma and produces conidiophores (Pl. LXXXI, D, e). In such a case new conidia might be produced before an extensive area of the host tissue had been killed. Usually, however, the fungus grows farther into the host before conidia are formed. It probably is true, as first suggested by Uzel (10), that the fungus causes asphyxiation and consequent collapse of the parenchyma cells, since only a slight intercellular growth of the fungus occurs. An attempt by the host cells to isolate the invading organism is seen in the massing of heavily staining substances (Pl. LXXXI E, f) in the parenchyma cells which adjoin the air chamber. Under certain conditions this isolation probably is accomplished and the host cells then remain turgid and normal. Where this can not be done, the cells surrounding the fungous mycelium collapse (Pl. LXXXI, G), the mycelium gradually produces tufts of conidiophores, and the characteristic leafspot is formed. The host under normal growth conditions is able to isolate this infected area, though as a result of severe, abundant infections, entire leaves may be covered with the conidiophore tufts of the fungus.

It then appears that there is no attractive force existing between the closed stomata and the conidial germ tubes of the fungus, and also that the latter do not possess enzymic power to directly penetrate the epidermal cells. However, with open stomata germ-tube penetration may occur, even though some length must be attained before the tube can

reach the pore opening. The reaction upon penetration induces a great change in the type of fungous growth, the fungous cells becoming large and round. It is to be concluded that since growth continues immediately in the air chamber below the stomata, the stomatal function of gaseous interchange is needed for the development of the mycelium in the host, as well as a force for initial penetration. It seems evident, therefore, that since germ-tube penetration may occur only when the stomata are open, and since stomatal movement is directly related to daylight hours, infection takes place only at this time.

#### SUMMARY

The study of the relation of stomatal movement to infection of the sugar-beet plant by *Cercospora beticola* Sacc. has revealed that certain morphological and environmental factors influence stomatal activity, and, in turn, the latter, together with a favorable growth of the fungus, influences infection.

Leaf maturity, light, temperature, and relative humidity are factors concerned with stomatal movement.

Leaf maturity may be determined by two characters which for any given stage have been found to remain uniform—i. e., the number of stomata present per square millimeter of leaf surface, and the length of the stomatal pore. These characters, taken together, give a good indication of leaf maturity, regardless of leaf size or position on the plant. Leaf maturity has a direct relation to stomatal activity in that movement is greater on mature than on young leaves, while on old leaves only very slight movement has been observed.

Light is probably one of the fundamental environmental factors that influence stomatal movement, and while direct sunlight may have an accelerating action, it is not essential for stomatal opening, since stomata may open widely in the shade.

Good stomatal opening has been obtained at temperatures ranging from 70° to 90° F. With these optimum temperatures active, relative humidity, with its associated causes and their effects, greatly influences stomatal movement. A high humidity favors stomatal opening, while a low humidity is associated with closure of the stomata. If the humidity remains above 60 through the day hours, the stomata will probably remain well open; but if it falls much below 50, stomatal closure will probably result.

Some of the factors influencing infection of beet leaves by *C. beticola* are rapidity of germ-tube growth, maturity of the leaves, and stomatal movement.

Fresh viable conidia of *C. beticola* germinate equally well and grow rapidly in distilled water, soil decoction, irrigation water, and bean decoction, in either darkness or diffused light at 24° C.



Infection, both artificial and natural, occurs best on mature leaves, and this is associated with the movement of the stomata.

Penetration of the leaf by the conidial germ tubes of *C. beticola* has been observed to occur only through *open* stomata, and consequently infection probably takes place during the day hours. An isolation of the invading organism is attempted by the leaf cells as soon as penetration occurs, but when this is not successful, the fungus by further growth produces a well-defined leafspot.

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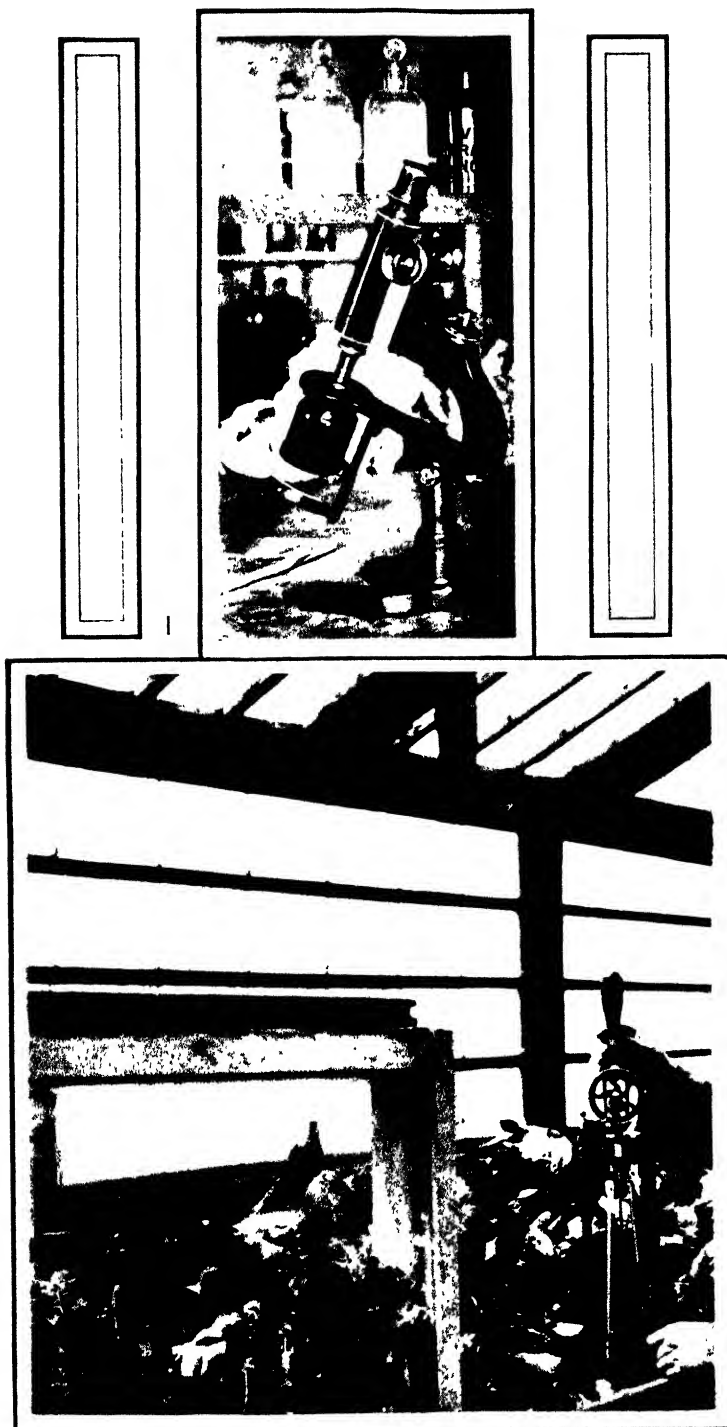
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PLATE LXXX

Fig. 1.—Stomatoscope designed by Dr. F. E. Lloyd and used for a part of these studies.

Fig. 2.—Humidity box in place over plants in the greenhouse for maintaining different relative humidities. Also a cog psychrometer used for checking hygrothermographs kept among the sugar-beet plants.



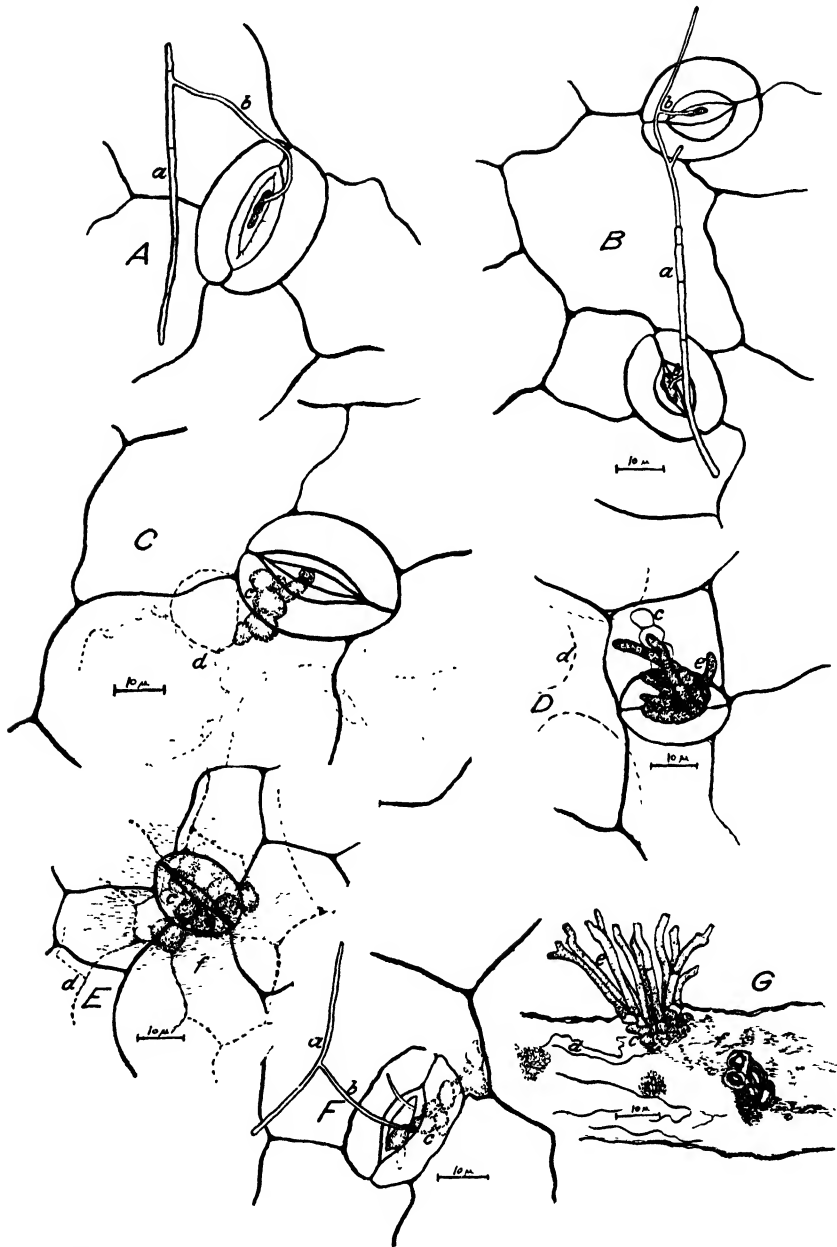


PLATE LXXXI

*Cercospora beticola* Sacc:

Fig. 1.—Conidia germinating on a sugar-beet leaf, with germ tubes entering open stomata. *A*, *a*, conidium; *b*, germ tube. *B*, *a*, conidium; *b*, *b*, two germ tubes penetrating two stomata. *C*, *c*, host mycelium below stoma in air chamber and forming a haustorium against a palisade parenchyma cell (*d*) represented with their chloroplasts by dotted lines. *D*, *c*, host mycelium in air chamber; *d*, parenchyma cells; *e*, exit of conidiophores. *E*, *c*, host mycelium; *d*, parenchyma cells; *f*, heavily staining host substance probably secreted for isolation purposes. *F*, *a*, conidium; *b*, germ tube; *c*, host mycelium in guard cell and epidermal cell. *G*, *c*, host mycelium or sclerotium; *d*, collapsed parenchyma cells; *e*, conidiophores; *f*, heavily staining host substance. (Camera-lucida drawings.)



## A METHOD OF CORRECTING FOR SOIL HETEROGENEITY IN VARIETY TESTS<sup>1</sup>

By FRANK M. SURFACE and RAYMOND PEARL,  
*Biologists, Maine Agricultural Experiment Station*

Men with practical experience in conducting variety tests and fertilizer experiments are free to admit that in many cases the results of ordinary field trials are of little or no value. The reason for this lies in the large number of factors which are beyond the control of the experimenter. In many instances variation in any one of these uncontrollable factors may influence the final results to a greater extent than the one controlled variable for which the experiment was undertaken.

On the other hand, field trials and variety tests play an important part in agricultural investigations. Such tests are an indispensable adjunct to plant-breeding work. The final test of new varieties or new strains must be made under field conditions. It is therefore of the greatest importance that methods should be devised which will in some measure at least take account of these uncontrollable factors.

No one of these factors is of more importance than the variation in the soil in different plots. It is practically impossible to secure for such field trials a tract of land that is absolutely uniform. The literature of variety tests abounds in illustrations of this fact.

In 1897 Larsen (8),<sup>2</sup> on the basis of results with timothy, reached the conclusion that more exact results were obtained where a given area was divided into a large number of plots than when it was divided into a few larger ones.

Holtsmark and Larsen (7) extended this idea and supplied additional evidence. Hall (1) in 1909 and Mercer and Hall (9) and Hall and Russell (2) in 1911 laid great emphasis upon soil heterogeneity in field tests. Among other things they did much to determine the most suitable sizes for experimental plots.

Montgomery (10, 11) has produced evidence showing that systematic repetition of plots over a given area reduces the variability in proportion to the number of repetitions; further, that while increase in the size of a plot decreases the variability up to a certain limit, a further increase in size is not attended by a corresponding decrease in variability.

As a result of these several investigations, it has become evident that much more reliable results are obtained by using several systematically repeated small plots than by using a single large one. This method is rapidly coming into more general use in field tests of all kinds. Never-

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<sup>1</sup> Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 93.

<sup>2</sup> Reference is made by number to "Literature cited," p. 1050.



theless, where for various reasons it is impossible to make a large number (10 to 20) of repetitions, the factor of soil heterogeneity still enters into the average yield. One or two exceptionally high or exceptionally low yields will unduly influence the average where the number of repetitions is only four or five.

In a series of papers Harris (3, 4, 5, 6) has called attention to various phases of the experimental error in field tests. In his most recent paper on this subject Harris (6) has proposed a method of measuring the heterogeneity of the soil of a field. The principle employed by Harris is stated thus (432-433):

If the irregularities in the experimental field are so large as to influence the yield of areas larger than single plots, they will tend to bring about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

This tendency to grouping of the high- and low-yielding plots is evident in most field experiments. It is clearly shown in the diagrams published by Montgomery (10).

The measure which Harris proposes for this heterogeneity (or homogeneity) of a field is the correlation between the yield of the ultimate small plots and the yield of various groups of contiguous plots. The more nearly this correlation approaches zero the more homogeneous the field. The more differentiated a given field is in regard to good and poor soil, the greater will be the value of the correlation coefficient.

This method of measuring the heterogeneity of a field is dependent somewhat upon the size of the ultimate plots and also upon the method of grouping. It does, however, mark a distinct advance in our method of dealing with small plot experiments.

While Harris's method provides a *measure* of the substratum heterogeneity in a given field, it does not provide any means of obtaining a corrective term for individual plots. While in field experiments it is of importance to know the amount of heterogeneity in the field as a whole, it is usually of much more importance to obtain some correction to apply to individual plots which will in some measure even up the differences in soil conditions.

The present paper is the result of an attempt to obtain such a corrective term. It is realized that the method proposed is far from ideal. It is believed, however, that it marks a step in this direction, and it is hoped that it may lead to further study of this important question.

The usual method of taking account of soil heterogeneity is the use of check plots. However, in very many cases this method has been far from satisfactory. It is not at all difficult to find examples in the literature of variety tests in which the amount of variation in the check plots is nearly or quite as great as the variation in the other varieties.<sup>1</sup> If check

<sup>1</sup> Davenport, Eugene, and Fraser, W. J. Experiments with wheat, 1888-1895. Experiments with oats, 1888-1895. Ill. Agr. Exp. Sta. Bul. 41, p. 147-160. 1896.

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plots are repeated at sufficiently frequent intervals, they will undoubtedly be a great aid in determining the correction for soil differences. However, where field tests of this kind are carried out on even a moderate scale, the use of check plots adds very materially to the labor and expense of the experiment. For example, in 1914 we grew 150 one-fortieth acre plots. From a study of the field it seems clear that any adequate system of checks would have required 1 check plot to every 5, or about 30 additional plots. The labor involved in handling these would have been considerable; and judging from the literature on the subject, the value of the results might still be very doubtful.

For several years this Station has been carrying on variety tests of oats. The object of these tests is to obtain some measure of the productiveness of new strains or varieties produced in the plant-breeding work. These new strains are always tested along with a number of standard commercial varieties. The method adopted in this work (13) is to grow four systematically repeated plots of each variety. The size of each plot is 33 feet square, or one-fortieth of an acre. The four plots thus make a total of one-tenth of an acre devoted to each variety. These plots have always been grown on a more or less rectangular piece of ground. (See fig. 4.) The fields for these tests have been chosen for their apparent uniformity. However, the resulting yields have always indicated that certain portions of the field were much better or worse in respect to soil fertility than the average of the field as a whole. In certain cases two or more of the four plots of a variety come to lie, say, in certain of these more fertile spots. This tends to produce an unduly high average for that variety.

In order to obtain a correcting value for these different soil conditions, it occurred to us to determine first the probable yield of each plot by the contingency method. This may be done as follows: Take a theoretical field divided into plots as in figure 1. Let  $a, b, c, \dots, l$  represent the observed yields of the respective plots, of which the mean yield is  $p$ . Then, assuming all plots to be planted with the same variety and conditions other than the soil to be uniform, we can obtain the most probable yield of, say, plot  $a$  by multiplying the sum  $ac$  by the sum  $aj$  and dividing by the total  $al$ . Proceeding in this way for each plot, we can obtain a calculated yield  $a', b', c', \dots, l'$  for each plot. The mean of these calculated yields will be the same as the mean of the observed yield—viz,  $p$ .

It is clear that these so-called calculated yields correspond to what Pearson (12) in his work on contingency has designated by  $\nu_{uv}$ , or the value for each square on the hypothesis of independent probability. The difference between the observed and calculated yields would then correspond to what Pearson calls a subcontingency

The "calculated" yields obtained by this contingency method represent the most probable yields of the respective plots based on the distribution of the observed yields. This method of estimating the probable

yield takes into account the soil differences in both directions across the field. To a certain extent it is dependent upon the assumption that the soil changes in a uniform manner from one side of the field to the other. Harris (6) has pointed out that this is not always the case, but that the diagrams of experimental fields indicate that differences in soil are more likely to occur as a spotting of the field. However, a closer study of the observed yields in many experimental fields indicates that there is a tendency for areas of good soil (high yield) to grade off through areas of medium soil to regions of poor soil. Ordinarily, the changes from one extreme to the other are not abrupt (see fig. 3, 4). The diagrams published by Montgomery (10) indicate this to some extent, although such diagrams do not show the graded changes as well as a study of the actual yields of contiguous plots.

a	b	c
d	e	f
g	h	i
j	k	l

FIG. 1.—Diagram illustrating the method of obtaining the "calculated" yield. (For explanation, see text.)

Further, if the distribution of the high and low "calculated" yields in figures 2 and 3 are compared with the high and low observed yields, it will be seen that the former show approximately the same "spotting" as the latter. This method does tend to lessen the variability and to smooth the results. While it is not ideal and does not obviate all the difficulties, it seems possible that this method may prove useful in estimating soil differences.

For cases like Montgomery's wheat experiment (10) or Mercer and Hall's field trials (9), where there are a number of plots all planted with the same variety, the contingency calculated yields may be used directly. For such experiments these calculated yields represent a smoothing of the original observations. In the case of field trials or variety tests, where different plots have different treatments or are planted with different varieties, such a smoothing tends to mask the actual differences between the plots. In such cases a further procedure is necessary.

In the case of a variety test the yield calculated by this contingency method may be regarded as the most probable yield of any given plot if we suppose the whole field had been planted with a single variety whose average yield was the same as the observed average of all the plots. The deviation of the calculated yield of a given plot from the mean of the field may be taken as a measure of the influence of the soil of that plot as compared with the whole field. Thus, if the calculated yield of a given plot is 10 bushels above the average of the field, it may be taken to mean that the soil on this plot is capable of producing 10 bushels more grain than the soil on the field as a whole.

This figure may be used to correct the observed yield of the corresponding plot. Thus, if the observed yield in a given plot is 80 bushels and the calculated yield is 5 bushels above the average of all the plots, then to make the yield of this plot comparable with the average of the field it would be necessary to reduce the observed yield by 5 bushels. Thus, we may obtain for this plot a "corrected" yield of 75 bushels.

Likewise, where the calculated yield is below the average, it is necessary to add a corresponding amount to the observed yield in order to take account of the deficiency in the soil of that plot.

Expressed in a formula, we may let  $O$  equal the observed yield and  $D$  the deviation of the calculated yield from the mean of the field. Then the

$$\text{"corrected" yield} = O - D$$

In fields where there are comparatively small differences between the yield of individual plots the direct method of correcting the yield as given above may be used. The corrected yields given in figures 2 and 3 were obtained by this direct method.

In the case of variety tests or experiments where there are likely to be marked differences between individual plots, it will be better to make corrections on a relative rather than an absolute basis. To do this, the deviation of the calculated yield from the mean of the field is determined as before. Next the percentage which each deviation is of the mean is determined. Then this percentage of the observed yield is added to, or subtracted from, the observed yield to obtain the corrected yield. An example will make this clear. Suppose the mean yield of the plots in a field is 70 bushels. The observed yield on a given plot is 80 bushels and the calculated yield of this plot is 77 bushels. Thus, the deviation of the calculated yield from the mean is +7 bushels, which is 10 per cent of the mean (70 bushels). The corrected yield will then be 10 per cent less than the observed; or 10 per cent of 80 equals 8 bushels. The resulting corrected yield will be 72 bushels. By the absolute method the corrected yield would have been 73 bushels. The corrected yields given in figure 4 and Table I have been obtained by this method.

It is next of importance to see whether this "corrected" yield has really obviated any of the difficulties. To test this, use may be made of the criterion of soil homogeneity proposed by Harris (6). This can best be tested upon such data as those furnished by the experimental fields of Montgomery (11) or Mercer and Hall (9).

Figure 2 is a diagram taken from Montgomery (11). It represents a field of Turkey wheat grown in 1908-9. This field was divided into 224 blocks (each 5.5 feet square), as indicated. The grain from each block

671	657	703	755	760	686	592	739	732	710	753	680	680	677	795	723
692	697	701	714	703	665	590	712	688	648	646	654	559	684	762	683
658	713	613	632	667	645	660	768	786	768	666	843	795	763	716	741
672	746	604	583	603	626	652	734	734	698	550	809	767	763	675	693
657	671	623	715	543	613	640	798	759	764	995	793	936	755	792	838
644	678	587	637	449	557	604	735	678	664	847	731	880	728	722	761
642	680	654	673	760	709	682	724	774	860	787	725	664	851	690	770
644	701	632	610	682	668	661	677	709	776	657	678	623	838	636	681
735	580	620	675	765	742	772	698	652	661	768	777	745	768	851	719
744	608	605	620	695	708	758	658	594	584	646	738	711	762	804	665
575	598	705	642	704	643	650	572	752	740	863	680	722	723	703	756
613	654	720	619	695	640	666	563	726	696	776	672	719	747	688	734
727	633	615	685	662	639	657	608	620	624	745	764	703	752	788	682
772	696	638	670	632	644	680	607	602	588	666	764	708	784	781	668
572	373	650	645	692	644	632	574	606	648	806	791	629	650	679	588
664	500	622	682	715	699	705	624	640	666	784	841	684	730	723	626
580	425	732	730	706	732	736	655	673	793	765	576	609	568	728	620
641	504	771	732	694	754	776	672	673	776	705	593	631	616	738	623
588	526	596	777	776	779	721	728	604	742	665	621	611	623	646	617
649	605	636	780	765	801	762	745	673	725	606	638	633	671	657	621
617	683	726	835	668	664	691	770	775	685	723	583	580	395	511	653
682	765	770	842	661	690	735	801	779	672	668	604	606	447	526	661
602	662	640	700	650	655	563	600	730	690	713	530	568	410	438	636
710	786	720	753	690	727	652	677	781	725	709	597	640	506	579	690
665	736	630	598	895	592	593	659	718	705	667	585	560	655	633	733
726	815	670	601	883	614	633	686	718	688	608	602	582	704	644	736
609	706	790	678	695	715	622	658	597	632	713	585	657	495	618	652
682	797	842	753	697	750	675	697	610	628	668	615	692	556	641	668

FIG. 2.—Diagram showing the observed and corrected yield (in grams) of grain on each of Montgomery's wheat plots in 1908-9. The upper figure in each plot is the observed yield and the lower the corrected.

was threshed and weighed separately. The upper figure in each square is the observed yield of grain in grams. The lower figure is the corrected yield obtained by the method outlined above. The mean yield of these plots is taken as 681 gm.

Figure 3 represents the combination plots obtained by grouping the plots in figure 2 in groups of four—i. e., a two- by two- fold grouping. In this figure the upper number in each plot is the observed yield, the lower number the corrected yield, while the middle number is the "calculated" yield. This latter is inserted to illustrate the method of obtaining the corrected yield. The mean yield of these grouped plots is taken as 2,723 gm.

Now, if we calculate the correlation between the observed yield of the ultimate plots and the observed yield of the combination plots it is found that

$$r = +0.358 \pm 0.039$$

This shows a fairly large coefficient of correlation, indicating a relatively large heterogeneity in the soil of this field.

If we calculate the correlation between the corrected yields of the ultimate plots and the corrected yields of the combination plots it is found that

$$r = +0.111 \pm 0.045$$

This coefficient is less than three times its probable error and is hardly to be regarded as significantly greater than 0. In any case it indicates that this method of correcting the yields has practically, if not quite, eliminated the influence of differences in soil of different plots.

2,699 2,616 2,806	2,703 2,826 2,600	2,758 2,894 2,587	2,759 2,798 2,684	2,996 2,953 2,766	2,942 3,007 2,658	2,915 2,766 2,872	2,975 2,887 2,811
2,650 2,707 2,666	2,665 2,924 2,464	2,625 2,994 2,354	2,844 2,895 2,672	3,157 3,056 2,824	3,300 3,112 2,911	3,206 2,862 3,067	3,090 2,987 2,826
2,488 2,594 2,617	2,642 2,802 2,563	2,854 2,869 2,708	2,692 2,774 2,641	2,805 2,928 2,600	3,088 2,982 2,829	2,958 2,743 2,938	3,029 2,863 2,889
2,305 2,414 2,614	2,505 2,608 2,020	2,637 2,670 2,690	2,471 2,583 2,611	2,498 2,725 2,496	3,106 2,776 3,053	2,734 2,553 2,904	2,737 2,664 2,796
2,119 2,444 2,398	2,835 2,640 2,928	2,993 2,703 3,011	2,840 2,614 2,949	2,812 2,759 2,776	2,627 2,809 2,541	2,411 2,584 2,540	2,611 2,697 2,637
2,564 2,345 2,942	2,901 2,533 3,091	2,637 2,593 2,767	2,624 2,508 2,839	2,880 2,647 2,956	2,549 2,694 2,578	1,953 2,479 2,197	2,278 2,587 2,414
2,716 2,421 3,018	2,696 2,615 2,804	2,897 2,677 2,943	2,532 2,589 2,666	2,652 2,732 2,643	2,550 2,782 2,491	2,367 2,559 2,531	2,636 2,671 2,688

FIG. 3.—Diagram showing the observed, corrected, and calculated yield (in grams) of Montgomery's wheat plots in groups of four, taken from figure 2.

Similar coefficients have been calculated for other fields with corresponding results.

It will next be of interest to test this method in the case of an actual variety test. This has been done in the case of all of our own variety test fields. The results will be published in another place in connection with a discussion of some pure-line oat varieties. In order to furnish an example of the use of this method in a variety test, the results of our 1915 test of oat varieties are given below.

Figure 4 represents a diagram of the 1915 plots of oats at the Highmoor Farm (Monmouth, Me.). In the upper left-hand corner of each square is the plot number as it occurs in our records. Immediately below this is the name of the variety. In the case of the pure-line varieties these are

indicated by our own record number—for example, as Maine 340, Maine 357, etc. The upper of the two remaining numbers in each square is the observed yield and the lower number is the corrected yield. All yields are given in bushels per acre.

905 Irish Victor 75.93 78.34	904 Maine 336 73.75 77.11	903 Siberian 72.12 72.02	902 Maine 230 75.25 76.42	901 Banner 73.75 74.85	900 Maine 351 77.75 79.34	899 Swedish Select 68.75 75.16	898 Maine 357 85.94 79.03
913 Maine 247 84.37 85.20	912 Senator 80.87 82.08	911 Maine 281 83.37 81.36	910 Maine 891 82.75 82.19	909 Minn. 26 90.93 90.27	908 Maine 340 83.12 82.60	907 Kherson 83.12 86.99	906 Maine 337 88.75 79.43
921 Maine 286 85.62 85.79	920 Early Pearl 85.00 86.38	919 Maine 346 69.38 67.14	918 Imported Scotch 70.31 69.26	917 Maine 307 83.37 75.70	916 Gold Rain 83.37 82.55	915 Maine 355 73.75 78.57	914 Prosperity 77.50 68.68
929 Maine 336 72.19 72.70	928 Siberian 80.62 82.33	927 Maine 230 74.62 72.61	926 Banner 90.31 89.45	925 Maine 351 78.37 77.59	924 Swedish Select 67.50 67.19	923 Maine 357 73.75 78.93	922 Maine 918 81.25 72.47
937 Senator 61.25 62.35	936 Maine 281 68.75 70.96	935 Maine 978 82.50 81.21	934 Minn. 26 80.25 80.38	933 Maine 340 84.00 84.10	932 Kherson 66.25 66.69	931 Maine 337 81.25 87.79	930 Irish Victor 87.50 79.12
945 Early Pearl 96.50 87.07	944 Maine 346 82.50 75.73	943 Imported Scotch 68.75 59.45	942 Maine 307 82.12 72.59	941 Gold Rain 86.87 76.74	940 Maine 355 93.12 82.83	939 Prosperity 83.37 81.05	938 Maine 247 90.62 70.24
953 Siberian 75.25 74.80	952 Maine 230 76.25 76.90	951 Banner 83.45 80.08	950 Maine 351 73.12 71.45	949 Swedish Select 65.25 63.74	948 Maine 357 82.12 80.67	947 Maine 982 85.62 90.59	946 Maine 286 85.62 75.13
961 Maine 281 81.25 82.94	960 Maine 1053 78.75 81.49	959 Minn. 26 81.25 80.23	958 Maine 340 75.31 75.64	957 Kherson 73.75 74.05	956 Maine 337 73.75 74.45	955 Irish Victor 61.87 67.01	954 Maine 336 84.06 76.27
969 Maine 346 76.87 83.56	968 Imported Scotch 64.65 71.12	967 Maine 307 77.50 81.83	966 Gold Rain 71.56 71.10	965 Maine 355 78.13 83.71	964 Prosperity 70.00 75.36	963 Maine 247 70.00 80.17	962 Senator 60.00 58.88
977 Maine 230 85.62 81.40	976 Banner 85.62 82.68	975 Maine 351 80.62 73.76	974 Swedish Select 71.87 67.08	973 Maine 357 76.87 71.70	972 Maine 1054 84.37 79.19	971 Maine 286 77.81 79.18	970 Early Pearl 90.87 75.35
985 Maine 1064 66.56 60.19	984 Minn. 26 74.37 80.13	983 Maine 340 89.00 91.86	982 Kherson 66.25 69.46	981 Maine 337 71.56 74.99	980 Irish Victor 76.25 80.31	979 Maine 336 65.94 74.10	978 Siberian 83.12 79.43
993 Imported Scotch 55.00 59.38	992 Maine 307 71.25 77.86	991 Gold Rain 77.50 81.23	990 Maine 355 83.75 89.13	989 Prosperity 67.12 71.41	988 Maine 247 75.62 80.83	987 Senator 51.25 58.34	986 Maine 281 91.87 89.39
76.74 78.38	76.74 79.45	76.74 75.81	76.74 77.13	76.74 77.09	996 Maine 286 61.88 62.50	995 Early Pearl 74.06 80.27	994 Maine 346 90.00 81.78

FIG. 4.—Diagram showing the yield of oats (in bushels per acre) on the 1915 variety-test field at Highmoor Farm (Monmouth, Me.) Each square represents a one-fortieth acre plot. (For description see text.)

In this field there were tested 11 commercial varieties and 12 pure-line varieties in quadruplicate one-fortieth acre plots. In addition, seven other pure lines were tested in single plots. It will be noted that in the lower row of the figures there are five plots not planted. In order to use this method of correction, it is necessary to assign values to these plots. The best method of doing this is to assign as the observed yield of each such plot the mean yield of the field. This method does not bias the results in either direction.

Table I shows the average yield, both observed and corrected, for the four plots of each commercial variety and for the 12 pure-line varieties. These corrected yields have been obtained by the percentage method described above.

TABLE I.—*Variation constants for the observed and corrected average yields of commercial and pure-line varieties of oats tested in 1915*

COMMERCIAL VARIETIES						
Variety.	Observed yield (bushels per acre).	Standard deviation.	Coefficient of variation.	Corrected yield (bushels per acre).	Standard deviation.	Coefficient of variation.
Minnesota No. 26 . . .	81.70±2.00	5.94±1.41	7.27±1.74	82.75±1.46	4.34±1.03	5.24±1.25
Early Pearl . . .	86.61±2.80	8.31±1.98	9.59±2.31	82.27±1.61	4.79±1.15	5.82±1.39
Banner . . .	83.28±2.03	6.04±1.44	7.25±1.73	81.77±1.77	5.26±1.25	6.43±1.53
Gold Rain . . . . .	79.83±1.84	5.48±1.30	6.86±1.64	77.90±1.50	4.48±1.06	5.73±1.37
Siberian . . . . .	77.78±1.45	4.33±1.03	5.57±1.33	77.14±1.34	4.00±.95	5.18±1.23
Irish Victor . . . . .	75.39±3.06	9.09±2.16	12.06±2.91	76.19±1.80	5.35±1.27	7.02±1.68
Prosperity . . . . .	74.59±2.14	6.37±1.51	8.55±2.05	74.13±1.56	4.65±1.10	6.27±1.50
Swedish Select . . . .	68.34±.80	2.39±.56	3.50±.81	68.29±1.41	4.20±1.00	6.15±1.47
Kherson . . . . .	64.68±2.50	7.43±1.77	11.46±2.76	66.79±2.10	6.25±1.49	9.36±2.25
Imported Scotch . . .	64.68±.63	1.88±.44	2.91±.69	64.80±1.83	5.43±1.29	8.38±2.02
Senator . . . . .	55.84±1.62	4.81±1.14	8.61±2.06	57.92±1.11	3.32±.79	5.73±1.37
Average . . . . .	73.89	5.64	7.63	73.63	4.73	6.48
PURE-LINE VARIETIES						
No. 340 . . . . .	82.77±1.65	4.90±1.16	5.92±1.41	83.55±1.94	5.76±1.37	6.89±1.65
No. 355 . . . . .	82.19±2.44	7.24±1.72	8.81±2.11	83.55±1.26	3.76±.89	4.50±1.07
No. 281 . . . . .	81.31±2.78	8.27±1.97	10.17±2.45	81.16±2.22	6.61±1.57	8.14±1.94
No. 337 . . . . .	78.83±2.27	6.76±1.61	8.58±2.06	79.17±1.79	5.34±1.27	6.74±1.61
No. 247 . . . . .	80.15±2.66	7.92±1.88	9.88±2.16	79.11±1.84	5.47±1.30	6.91±1.65
No. 357 . . . . .	79.67±1.58	4.70±1.12	5.90±1.41	77.58±1.16	3.47±.82	4.47±1.06
No. 230 . . . . .	77.94±1.50	4.47±1.06	5.73±1.37	77.58±1.05	3.15±.74	4.02±.95
No. 346 . . . . .	79.69±2.54	7.50±1.80	9.49±2.28	77.04±2.16	6.41±1.52	8.32±1.99
No. 307 . . . . .	76.94±1.30	3.86±.92	5.02±1.20	77.00±1.73	3.36±.78	4.36±1.04
No. 286 . . . . .	77.73±3.26	9.69±2.31	12.47±3.01	75.65±2.86	8.49±2.02	11.22±2.70
No. 351 . . . . .	77.47±.91	2.73±.65	3.52±.84	75.54±1.04	3.10±.73	4.10±.97
No. 336 . . . . .	73.99±2.19	6.51±1.55	8.79±2.11	75.05±.58	1.74±.41	2.32±.55
Average . . . . .	79.06	6.22	7.86	78.50	4.72	6.00

From figure 4 it is seen that in many plots the corrected yield varies quite widely from the observed. However, Table I shows that when the four plots of each variety are averaged there are in most cases comparatively slight differences between the two. This point is a strong argument for the efficiency of four systematically repeated plots in reducing the experimental error. There are, however, a few cases in the table



where the corrected average yield is markedly different from the observed. An instance of this is seen in the Early Pearl variety (Table I). The observed average yield of this variety (86.6 bushels) was the highest obtained in 1915. The difference between the yield of this and the Minnesota No. 26 was nearly 5 bushels. The corrected average yield of these two varieties is practically the same, differing only in a fraction of a bushel. By referring back to figure 4 it is found that the high average yield of the Early Pearl was largely due to the influence of two plots, Nos. 945 and 970. These two plots happened to lie in exceptionally good soil. Their observed yields of 96.5 and 90.9 bushels per acre were reduced to the corrected yields of 87 and 75.4 bushels, respectively.

As is to be expected, the corrected average yields show in nearly all cases a much lower variability. This is true of both the absolute and relative variability. In one or two instances, as the Imported Scotch (Table I), the variability is greater in the case of the corrected yield. If all the varieties (Table I) are taken, the corrected yields will show an average decrease in the coefficient of variation of about  $1\frac{1}{2}$  per cent.

The table shows that with systematically repeated plots the yields corrected by this method do not differ radically from the actually observed yields. Such changes in the order of yield as do occur we believe more truly express the relative value of these varieties. This statement is based on the experience of several years with these same varieties.

In using this method attention should be called to one or two points. In the first place where a field of plots is very large or where it is relatively long and narrow better results will usually be obtained by breaking it up into smaller blocks for calculation. For example, our 1914 test field was 6 plots wide and 28 plots long. More satisfactory results were obtained by breaking this up into three blocks, two of which were 9 plots long, the other 10 plots. Each block was calculated as a separate field. In doing this, care should be taken that the blocks are not so small as to be unduly affected by a possible preponderance of very good or very poor varieties.

Another point to be remembered in the practical use of this method is that it can not be used to take account of uneven seeding, ravages of birds, or other irregularities in certain plots. Corrections, if any, for these factors should be added before employing the above method.

#### SUMMARY

It is generally admitted that field trials, including variety tests, are often of very little value because of the large number of uncontrollable factors. Nevertheless, field trials are becoming more and more a necessity in many phases of agricultural investigation.

Within recent years a number of investigators have shown that the experimental error in such trials can be greatly reduced by the use of

systematically repeated plots. Nevertheless, if the number of repetitions is not large, certain experiments may still be unduly influenced by irregularities in the field. It would therefore be desirable if some method could be devised by which the yields of individual plots could be corrected in such a way as to take account of these irregularities.

Check plots have frequently been used for this purpose. But, aside from the extra labor and expense involved, the results from check plots have been far from satisfactory in many cases.

In the present paper a method is proposed for use in correcting for differences in the soil of different plots. The method in its present form is adapted for use only when the plots are arranged in blocks similar to those in figure 4. The method of obtaining this correction factor is as follows: In the first place the probable yield of each plot is obtained by the contingency method. This "calculated" yield represents the most probable yield of each plot on the supposition that they have all been planted with a hypothetical variety whose mean yield is the same as the observed means of the field.

This "calculated" yield may then be used as a basis for determining a correction factor. If the calculated yield of a given plot is above the mean of the field it must be taken that the soil of this plot is better than the average of the field and a corresponding amount must be deducted from the observed yield. Likewise, if the calculated yield is below the average, a proportional amount must be added to the observed yield in order to make the plots comparable.

Still more comparable results will be obtained if the correction factors are based upon the percentage of the mean rather than upon the absolute figures.

Tests of the efficiency of this method by means of the measure of soil heterogeneity proposed by Harris (6) show in all cases a very marked reduction in the amount of heterogeneity when the corrected figures are used. When tested on our own experimental plots, this method leads to results which from other evidence, we have reason to believe, more nearly represent the truth than do the uncorrected yields.

It is realized that this method is not ideal and does not obviate all the difficulties connected with soil differences in plot experiments. It is hoped that this method may prove useful in certain kinds of plot experiments and that it may lead to further study of this problem.

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## FLOW THROUGH WEIR NOTCHES WITH THIN EDGES AND FULL CONTRACTIONS<sup>1</sup>

By V. M. CONE,

*Irrigation Engineer, Office of Public Roads and Rural Engineering*

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### INTRODUCTION

The developments in irrigation agriculture in the arid West have caused many changes to be made in the method of delivering water to canals and to individual irrigators. The value of water increases with the increase of irrigated acreage, and the long-accepted practice of fixing the charges for water on a per-acre-per-annum basis is rapidly losing ground in favor of charges based on the volume of water delivered. When irrigators pay according to the amounts of water used, there is every incentive for them to study the water requirements of their crops and to use the least quantities they judge to be necessary. This leads to a proper economy in the use of water, permits a greater acreage to be irrigated with the available water supply, and conserves the land.

The transition from a flat rate to a rate based on the water actually used is calling for a better knowledge of the accuracy and practicability of existing measuring devices as well as the development of new devices. The weir is generally considered an accurate device for measuring water, and it doubtless is such, provided it is properly installed and the correct formula is used for determining the discharge through the notch. Weirs constitute a large proportion of the devices in use for measuring irrigation water at the present time, being principally of the rectangular notch

<sup>1</sup> This paper is based on experiments conducted in the hydraulic laboratory at Fort Collins, Colo., under cooperative agreement between the Office of Experiment Stations of the United States Department of Agriculture and the Colorado Agricultural Experiment Station.

or Francis type, and the Cipolletti type. Most of the weirs in use have notches with crest lengths of 4 feet or less, being such as are adapted to the delivery of water for farm units. Unfortunately, owing chiefly to the confusion of the statements contained in the literature on weirs, various standards of dimensions have been used in the construction of the weirs now in use. This lack of uniformity results in many erroneous measurements.

The basic experiments with notches having thin edges and full contractions were made by James B. Francis (5)<sup>1</sup> from 1848 to 1852. These have subsequently been enlarged upon by several experimenters and mathematicians. Francis made three series of experiments with rectangular-notch weirs, but the discharges were measured directly in only one series (5, p. 75-76). In each of the two other series an equal flow of water was made to pass through notches of different lengths, the crest lengths and the heads being noted. In the experiments, where the discharges were measured volumetrically, only notches of approximately 8- and 10-foot lengths were used, and the heads ranged from only 7 to 19 inches (5, p. 122-125). Most of the experiments were made with the 10-foot notch, as they were to be applied directly to the measurement of water for power purposes. Francis stated (5, p. 133) that the formula which he derived would apply to heads ranging from 6 to 24 inches, but in no case was it to be used either for heads exceeding one-third the length of the crest or for very small heads. With these limitations the formula can not be used for weirs having crest lengths of less than 1.5 feet nor for heads exceeding 2 feet. For a 1.5-foot crest the formula can be used only for a 0.5-foot head. Horton states (7) that the Francis data and formula will hold for heads from 0.5 foot to 4 feet. Francis's experiments were very carefully and conscientiously made, but were with longer notches and greater volumes of water than are usually needed in delivering water to irrigators. The Francis formula is frequently used, however, without regard to the limits which he imposed upon it, and it is not uncommon to see tables computed from it that give discharges for heads as low as 0.01 foot, with heads as high as 1 foot for a crest length of 1 foot, and for crest lengths varying from 0.5 foot to 20 feet.

The most popular weir notch has been the trapezoidal type with side slopes of one horizontal to four vertical. This type was designed and the formula deduced by the Italian engineer Cesare Cipolletti (3), with the idea of automatically eliminating the correction for end contractions necessary with the rectangular notches and thus obtaining a type of notch the discharge through which would be proportional to the length of the crest and free from error in excess of one-half of 1 per cent from any single cause. Cipolletti derived the shape of the notch by a mathematical modification of the Francis formula for the rectangular notch.

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<sup>1</sup> Reference is made by number to "Literature cited." p. 1110-1112.

He obtained the values for the coefficient and exponent by examining Francis's experimental data and increasing Francis's coefficient value somewhat arbitrarily by 1 per cent. He also made a few experiments, but stated that his formula was subject to the limitations imposed by Francis; consequently the extension of the range of application of the formula has been an excursion into unexplored territory. The notch designed by Cipolletti was intended to measure a minimum discharge of 150 liters (5.3 cubic feet) per second and a maximum discharge of 300 liters (10.6 cubic feet) per second, thus further restricting the use of the Cipolletti formula to notches having crest lengths of not less than 3 feet nor more than 8 feet.

There is great practical need in irrigation practice for weirs with small notches and for measurements with small depths of water over the crests of the notches. It also is important to know that the discharge formulas are correct, as many other forms of measuring devices are commonly calibrated by being hitched in tandem with the weir. For these reasons it was deemed advisable to conduct a series of experiments with notches having thin edges and full contractions (1) to determine whether the Francis and Cipolletti formulas hold for notches of the sizes ordinarily used in irrigation practice and (2), in case the old formulas did not hold, to derive new formulas.

#### LABORATORY EQUIPMENT AND METHODS

The hydraulic laboratory at Fort Collins was built in 1912-13, under a cooperative agreement between the Office of Experiment Stations, United States Department of Agriculture, and the Colorado Agricultural Experiment Station, and is designed for research work in hydraulics, especially gravity flow.<sup>1</sup> With the exception of the building, which is of brick, the laboratory is constructed almost entirely of concrete and metal to give it rigidity, permanency, and water-tightness. All water faces of concrete are covered with a 3 to 1 cement-plaster coat three-eighths of an inch thick. Tests have shown the seepage losses to be negligible. The plan and a sectional elevation of the laboratory are shown in figure 1. The circular storage reservoir has a top diameter of 87 feet, side slopes of 1 to 1, and is  $6\frac{1}{2}$  feet deep. The headrace connecting it with the weir box is approximately 60 feet long, 4 feet deep, and 6 feet wide for the first 15 feet below the head gates and then expands to 6 feet deep and 10 feet wide at the weir box. The weir box is 20 feet long, 10 feet wide, and 6 feet deep, and has a heavy T-iron frame approximately 3 feet high and 6 feet long in its bulkhead wall. This frame is surfaced, bored for  $\frac{3}{8}$ -inch bolts, and so arranged that the plates containing or forming the notches or orifices and other measuring devices requiring a vertical position can be adjusted accurately for experiments. The joints between the plates and the frame are made

<sup>1</sup> For a complete description of the hydraulic laboratory, see an earlier article by the writer (4).

water-tight by flat rubber gaskets. The water passing through the notches or orifices falls into a concrete spill box 4 feet deep, 10 feet wide, and 9 feet long, which is connected with an auxiliary or waste reser-

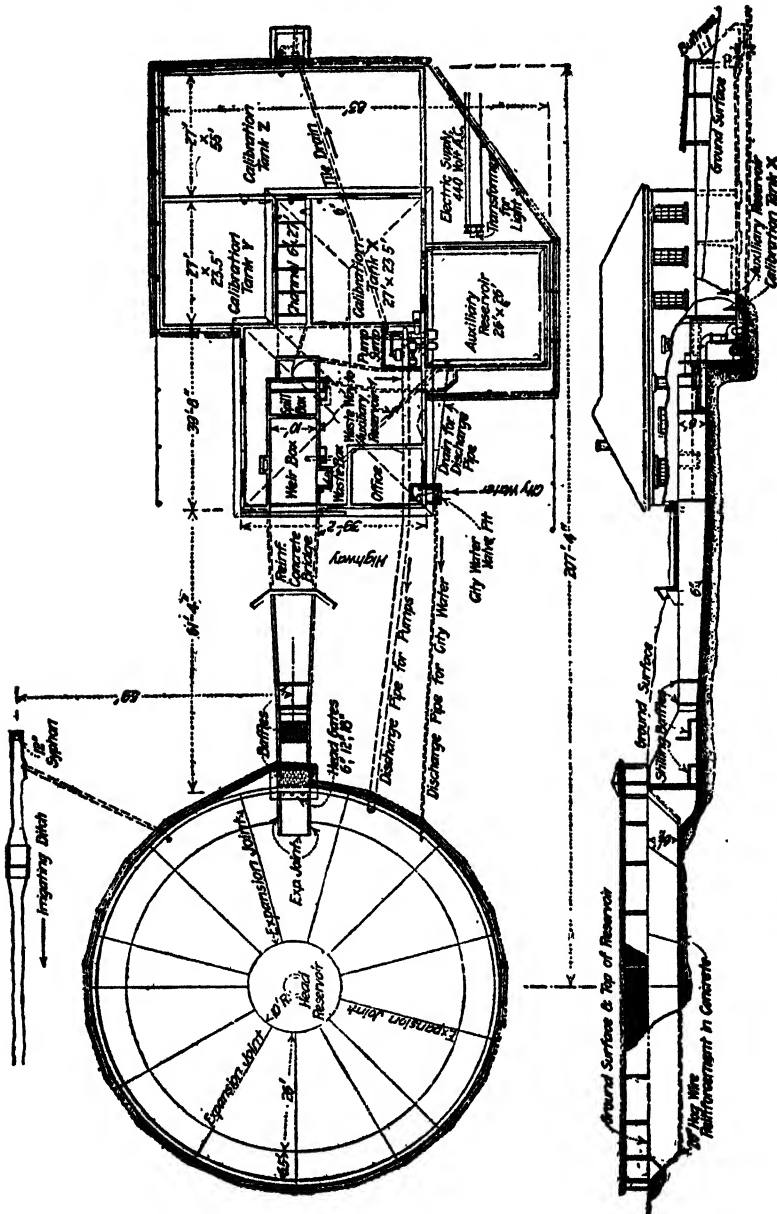


FIG. 1.—Plan and sectional elevations of the Fort Collins hydraulic laboratory.

voir by one channel and with the calibrated tanks by another. The two 22-inch circular openings leading to these channels are separated by a steel plate, and a single disk on the lever arm makes a double shear

gate for the openings. The calibrated tanks and the wasteways on the weir box as well as the spill box are connected with the waste reservoir, from which the water can be returned to the storage reservoir by either a 12-inch or a 5-inch horizontal centrifugal pump driven by electricity. The floors of the calibrated tanks and the waste reservoir are 19 feet lower than the coping of the storage reservoir.

Some of the means used to secure accuracy in the experiments are as follows: The laboratory is so arranged that the centers of the storage reservoir, the headrace, the frame in the end of the weir box, and the channel from the spill box to the calibrated tanks all lie in the same straight line, thus permitting the water to approach and leave the device under experiment in a straight line.

The three head gates between the storage reservoir and the headrace—6, 12, and 18 inches in diameter, respectively—permit a fairly accurate regulation of the water entering the weir box.

Immediately below the head gates a series of two horizontal and two vertical baffles breaks up the eddy currents and reduces pulsations and wave action to such an extent that the water, before entering the weir box, is in a pondlike condition.

In one side of the weir box, about 15 feet upstream from the bulkhead, is an overpour spillway which resembles a door 2 feet high and 3 feet long hinged at the bottom. The top of this spillway when in an upright position is slightly below the top of the weir box. Aprons of oiled canvas attached to the sides of the weir box and to the face of the door prevent leakage and compel the water to pass over the crest of the spillway. A 4-inch gate valve placed at the side of the spillway permits a still more careful regulation of the depth of the water in the weir box. Both the spillway and the gate valve can be adjusted by the hook-gauge observer on the opposite side of the weir box by means of screw controls operated by handwheels placed on the ends of long rods. By always having some water running over the spillway it was possible to keep the head upon the device under test constant throughout the duration of the experiment, usually from 20 to 40 minutes, depending upon the volume of water being run.

The elevations of the water in the weir box and the spill box are observed in concrete gauge boxes built on the outside walls of the respective boxes. These gauge boxes are 1 foot by 2 feet by 4 feet deep, inside dimensions, and the water enters each of them through four 1-inch pipes. The gauge box for the weir box is located 10 feet upstream, and that for the spill box 7 feet downstream from the bulkhead. The pipes leading to the latter, however, take water from the spill box at a point only  $3\frac{1}{2}$  feet downstream from the plane of the weir. Each gauge box is equipped with an electric drop light and a Boyden hook gauge anchored in the concrete wall, and readings of the water level can be made to 0.001 of a foot.



In order to refer the elevation of the crest of the notch being experimented with to a reading of the weir-box hook gauge to the nearest 0.001 foot, the instrument shown in figure 2 was devised. The ends of the legs and the hook can be adjusted so as to make the distance from the top of the plate to the groove in the legs exactly equal to the distance from the top of the plate to the point of the hook. By resting the notched legs on the crest of the notch and adjusting the plate to a horizontal position with a sensitive level, the point of the hook is brought to the same elevation as the crest of the notch. Water is run into the weir box, and the surface of the water is adjusted to the point of the crest-hook gauge. Since it is possible to maintain the water level in the weir box quite accurately, the hook-gauge reading in the weir-box gauge box is taken to correspond to the crest elevation of the notch. Repeated determinations of this nature indicated a high degree of accuracy.

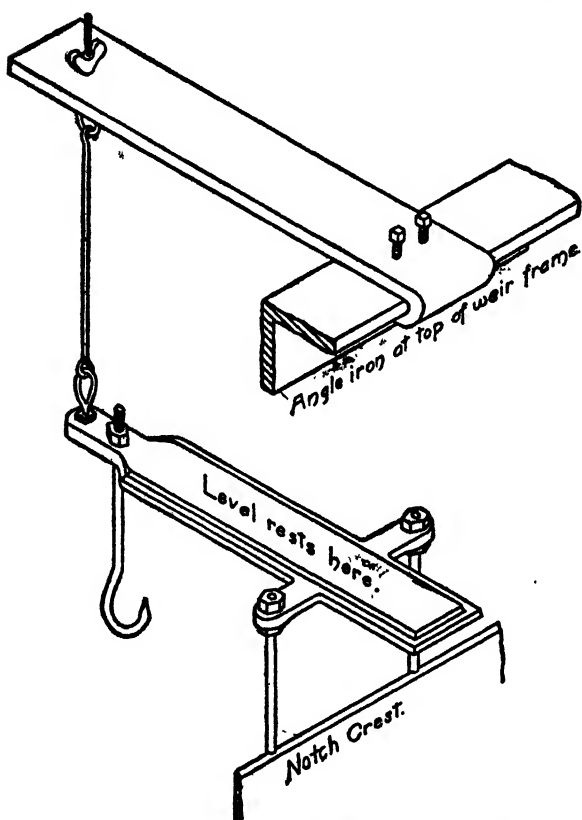


FIG. 2.—Device used in referring elevations of the notch crest to the reading of the hook gauge.

tests are being started or stopped, means had to be provided for quickly turning the flow into the channel to the calibrated tanks when the desired conditions for the test had been obtained. This is accomplished by means of the double shear gate used to close the two 22-inch circular openings in the spill box. The lever arm of this gate is 8 feet long, the disk is seated by means of steel shear springs, and the gate is positive and instantaneous in action. When the gate handle reaches midpoint of its swing, it strikes a gong, which is a signal to the hook-gauge observer to start or stop the stop watch used in recording the

In order to avoid the fluctuating conditions of the flow which occur when

duration of the experiments. The error in time in operating the shear gate and the stop watch is only a small fraction of a second.

The calibrated tanks cover an area 55 feet square, divided by 12-inch vertical-sided concrete walls into one tank 27 by 55 feet, two tanks each  $23\frac{1}{2}$  by 27 feet, and a channel 6 by 27 feet, which is connected with each tank by a 14-inch circular orifice placed on the floor line and controlled by a gate. The tanks are  $8\frac{1}{2}$  feet deep. Their floors are all at the same elevation, and they have a combined capacity of more than 22,000 cubic feet available for experimental purposes. The tanks have been carefully calibrated, corrections having been made for all irregularities, gate openings, rods, etc., and tables have been prepared giving the capacity at each 0.001 foot in elevation. A brass rod 1 inch in diameter and 9 feet long was placed in a vertical position near one corner in each calibrated tank, being held out from the wall about 6 inches by iron brackets set in the concrete (fig. 3). Holes drilled in these rods at carefully measured intervals of about 18 inches serve as datum points when the quantity of water in the tanks is being measured. The elevation of the water in the tanks is determined to 0.001 foot by means of a hook gauge having fixed to its back a heavy clamp provided with a pin which fits snugly into the holes in the rod. A steel ladder was placed adjacent to the brass standard rods in each tank and anchored to the concrete. The platform shown in figure 3 is 20 by 24 inches and can be lowered close to the water surface and secured to any of the ladders by means of hooks. The funnel-shaped arrangement attached to the platform has a  $\frac{1}{4}$ -inch hole in the bottom and can be adjusted so as to form a stilling basin for the hook gauge. With the water levels at the beginning and the end of the experiment determined by means of the standard rod and hook gauge, the volume run during the experiment can be determined readily from the calibration tables.

Unless otherwise stated, the experiments recorded in this publication were made with notches the edges of which were one-sixteenth inch or less in thickness. The notch plates used were constructed either entirely of brass or of steel with brass notch edges. The crests and sides of the notches were dressed to true angles and straight lines, and by means of a micrometer caliper were calibrated to an allowable divergence of 0.002 inch from a straight line. The triangular notches were dressed to templates. The plate containing the notch under observation was placed in a vertical position in the T frame in the bulkhead of the weir box, and the crests of rectangular and Cipolletti notches were leveled to within 0.001 foot by means of a 12-inch steel-frame level, upon which a bubble division indicated a variation of 0.0004 foot for a length of 1 foot. The inner face of the bulkhead was flush with the crest of the notch. The triangular notch plates were placed so that a vertical line would bisect the angle formed by the sides of the notch. In all the experi-

ments except those upon the effect of contraction (p. 1091) the bottom of the weir box was approximately  $4\frac{1}{2}$  feet below the crests of the notches, and the sides of the weir box were 3 to  $4\frac{1}{2}$  feet from the ends of the crests, depending upon the size of the notches. In all the experiments the floor

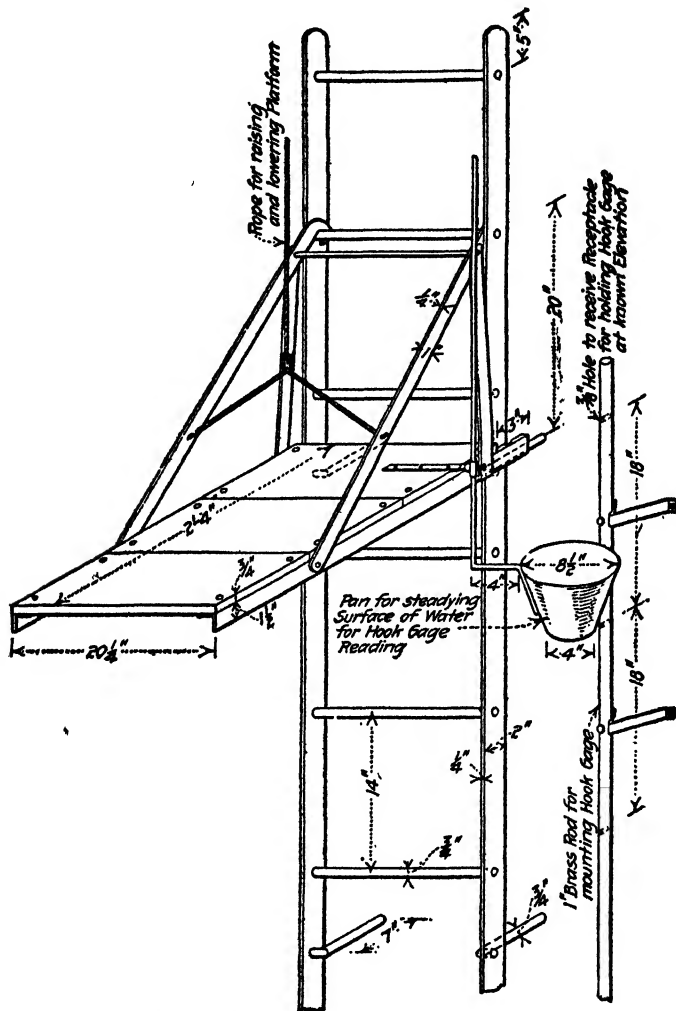


FIG. 3.—Ladder, platform, and datum rod used in calibration tanks.

of the spill box was approximately 4.5 feet below the vertex, or crest, of the notch.

Thirty or forty tests were made upon each notch, the experimental variable being the head. Intervals of head of 0.05 foot were used, and duplicate tests were run for each 0.1 foot of head. If the data from the duplicate tests did not agree within one-half of 1 per cent, the tests were

repeated until such agreement was obtained. It is not claimed that this arbitrary rule insures the accuracy of results of the individual tests, but it did lead to the detection of irregularities in the working conditions and increased the probability of accuracy. Comparatively few tests had to be rerun, which indicates the stability of the experimental tests and the nice control of the heads made possible by the head gates, wasteways, and baffles.

The heads and the corresponding discharges obtained were plotted for the various notches. The curves were then drawn which best represented the discharges through the different notches, the plottings being made upon such a scale that discharge values could be read from the curves to three decimal places.

The following method was used in smoothing the curves and obtaining the values for  $C$  in the general formula  $Q = CLH^n$ :

Discharge values were taken from the curves for each 0.05 foot head, and the slope was determined for each straight line connecting pairs of points. The slope for each point was first taken as the average between the slopes of the two straight lines to which it was common; then, calling the point in question  $b$ , the point for the next 0.05 foot head above,  $a$ , and that below,  $c$ , the slopes were given a second smoothing by the equation  $\frac{a + 2b + c}{4} = b$ ; and a third smoothing was obtained by substituting the values obtained by the second smoothing in the equation  $\frac{a + 2b + 3c + 2d + e}{9} = c$ . These values were plotted, and the equation of the resulting curve was used to compute the last smoothing of the slopes. Substituting these computed values for  $n$  in the general formula  $Q = CLH^n$ , the corresponding value of  $C$  was obtained for each head.

#### EXPERIMENTS WITH NOTCHES HAVING FREE FLOW

##### DEDUCTIONS OF FORMULAS FOR RECTANGULAR AND TRAPEZOIDAL NOTCHES

The general type of formula heretofore used for discharges through rectangular and trapezoidal notches is  $Q = CLH^n$ , in which  $L$  is length of crest,  $H$  the head of water over the crest, and  $C$  and  $n$  are constant for each type of weir. Expressed logarithmically, the general formula becomes  $\log Q = \log C + \log L + n \log H$ , which equation, when plotted, gives a straight line whose slope is  $n$  and whose intercept is  $\log C + \log L$ .

The data obtained for the rectangular and Cipolletti notches, when plotted logarithmically, gave curves instead of straight lines. It was found, however, that a general straight-line equation could be deduced for the discharges through the rectangular notches, which, within the range of the experiments, would give discharges as close to the experi-

mental data as would the general curve equation. The experimental data indicate, however, that the general curve equation would hold true for a greater range of notch lengths and heads than would the general straight-line equation. Table I, for the Cipolletti notches, gives the discharge values for the different heads as read from the curve, the experimental discharge values (observed discharges) at greatest variance with the curve discharge values, and the values of the exponents  $n$  and coefficients  $C$  necessary in the Cipolletti formula to give the discharges obtained in the experiments. The values of  $n$  and  $C$  in the table show that the discharges for any notch, if plotted logarithmically, would not give a straight line, since neither the  $n$ 's nor the  $C$ 's are constant. A comparison of the curve discharge values and the observed discharges in the table also serves to indicate the accuracy of the experimental data. The variations of the  $n$ 's and  $C$ 's also hold for the rectangular notches, but are not so pronounced as in the case of the Cipolletti notches, since the discharge curves for rectangular notches are flatter.

TABLE I.—Discharges through Cipolletti notches, and the exponents and coefficients necessary in using the Cipolletti formula

Head.  Feet.	0.5066-foot notch.				1.0093-foot notch.				1.5088-foot notch.				2.0082-foot notch.				3.0011-foot notch.				4.0086-foot notch.			
	Discharge, cubic feet per second.		(n)	(C)	Discharge, cubic feet per second.		(n)	(C)	Discharge, cubic feet per second.		(n)	(C)	Discharge, cubic feet per second.		(n)	(C)	Discharge, cubic feet per second.		(n)	(C)	Discharge, cubic feet per second.		(n)	(C)
0.15	0.149	1.530	3.492		0.300	0.300	1.498	3.377	0.459	0.459	1.486	3.309	0.603	0.603	1.466	3.255	0.909	0.909	1.473	3.217	1.200	1.200	1.470	3.205
0.20	0.207	1.565	3.723		0.400	0.400	1.517	3.424	0.589	0.589	1.499	3.353	0.800	0.800	1.480	3.307	1.100	1.100	1.486	3.263	1.400	1.400	1.476	3.232
0.25	0.265	1.600	3.911		0.500	0.500	1.536	3.510	0.750	0.750	1.513	3.413	1.000	1.000	1.495	3.353	1.300	1.300	1.501	3.363	1.600	1.600	1.481	3.201
0.30	0.323	1.636	4.066		0.600	0.600	1.555	3.558	1.000	1.000	1.530	3.469	1.300	1.300	1.511	3.381	1.600	1.600	1.528	3.363	1.900	1.900	1.487	3.187
0.35	0.380	1.671	4.174		0.700	0.700	1.574	3.608	1.250	1.250	1.546	3.446	1.600	1.600	1.521	3.416	1.900	1.900	1.544	3.376	2.200	2.200	1.493	3.165
0.40	0.438	1.706	4.254		0.800	0.800	1.593	3.653	1.500	1.500	1.553	3.488	1.900	1.900	1.532	3.417	2.200	2.200	1.559	3.380	2.500	2.500	1.499	3.139
0.45	0.495	1.743	4.284		0.900	0.900	1.612	3.693	1.750	1.750	1.566	3.500	2.200	2.200	1.542	3.417	2.500	2.500	1.574	3.384	2.800	2.800	1.504	3.116
0.50	0.552	1.780	4.303		1.000	1.000	1.631	3.726	2.000	2.000	1.579	3.508	2.500	2.500	1.553	3.424	2.800	2.800	1.584	3.388	3.100	3.100	1.510	3.091
0.55	0.609	1.817	4.318		1.100	1.100	1.650	3.759	2.250	2.250	1.592	3.522	2.800	2.800	1.563	3.432	3.100	3.100	1.605	3.391	3.400	3.400	1.515	3.067
0.60	0.666	1.854	4.332		1.200	1.200	1.669	3.792	2.500	2.500	1.605	3.533	3.100	3.100	1.573	3.441	3.400	3.400	1.625	3.394	3.700	3.700	1.521	3.043
0.65	0.723	1.891	4.346		1.300	1.300	1.688	3.825	2.750	2.750	1.618	3.544	3.400	3.400	1.584	3.450	3.700	3.700	1.645	3.397	4.000	4.000	1.526	3.019
0.70	0.780	1.928	4.359		1.400	1.400	1.707	3.858	3.000	3.000	1.631	3.555	3.700	3.700	1.595	3.459	4.000	4.000	1.665	3.400	4.300	4.300	1.531	2.995
0.75	0.837	1.965	4.372		1.500	1.500	1.726	3.891	3.250	3.250	1.644	3.566	4.000	4.000	1.606	3.468	4.300	4.300	1.685	3.403	4.600	4.600	1.536	2.971
0.80	0.894	2.002	4.385		1.600	1.600	1.745	3.924	3.500	3.500	1.657	3.577	4.300	4.300	1.617	3.477	4.600	4.600	1.705	3.406	4.900	4.900	1.541	2.947
0.85	0.951	2.039	4.398		1.700	1.700	1.764	3.957	3.750	3.750	1.670	3.588	4.600	4.600	1.628	3.486	4.900	4.900	1.725	3.409	5.200	5.200	1.546	2.923
0.90	1.008	2.076	4.411		1.800	1.800	1.783	3.990	4.000	4.000	1.683	3.599	4.900	4.900	1.639	3.495	5.200	5.200	1.745	3.412	5.500	5.500	1.551	2.899
0.95	1.065	2.113	4.424		1.900	1.900	1.802	4.023	4.250	4.250	1.696	3.610	5.200	5.200	1.650	3.504	5.500	5.500	1.765	3.415	5.800	5.800	1.556	2.875
1.00	1.122	2.150	4.437		2.000	2.000	1.821	4.056	4.500	4.500	1.709	3.621	5.500	5.500	1.661	3.513	5.800	5.800	1.785	3.418	6.100	6.100	1.561	2.851
1.05	1.179	2.187	4.450		2.100	2.100	1.840	4.089	4.750	4.750	1.722	3.632	5.800	5.800	1.672	3.522	6.100	6.100	1.805	3.421	6.400	6.400	1.566	2.827
1.10	1.236	2.224	4.463		2.200	2.200	1.859	4.122	5.000	5.000	1.735	3.643	6.100	6.100	1.683	3.531	6.400	6.400	1.825	3.424	6.700	6.700	1.571	2.803
1.15	1.293	2.261	4.476		2.300	2.300	1.878	4.155	5.250	5.250	1.748	3.654	6.400	6.400	1.694	3.540	6.700	6.700	1.845	3.427	7.000	7.000	1.576	2.779
1.20	1.350	2.298	4.489		2.400	2.400	1.897	4.188	5.500	5.500	1.761	3.665	6.700	6.700	1.705	3.549	7.000	7.000	1.865	3.430	7.300	7.300	1.581	2.755
1.25	1.407	2.335	4.502		2.500	2.500	1.916	4.221	5.750	5.750	1.774	3.676	7.000	7.000	1.716	3.558	7.300	7.300	1.885	3.433	7.600	7.600	1.586	2.731
1.30	1.464	2.372	4.515		2.600	2.600	1.935	4.254	6.000	6.000	1.787	3.687	7.300	7.300	1.727	3.567	7.600	7.600	1.905	3.436	7.900	7.900	1.591	2.707

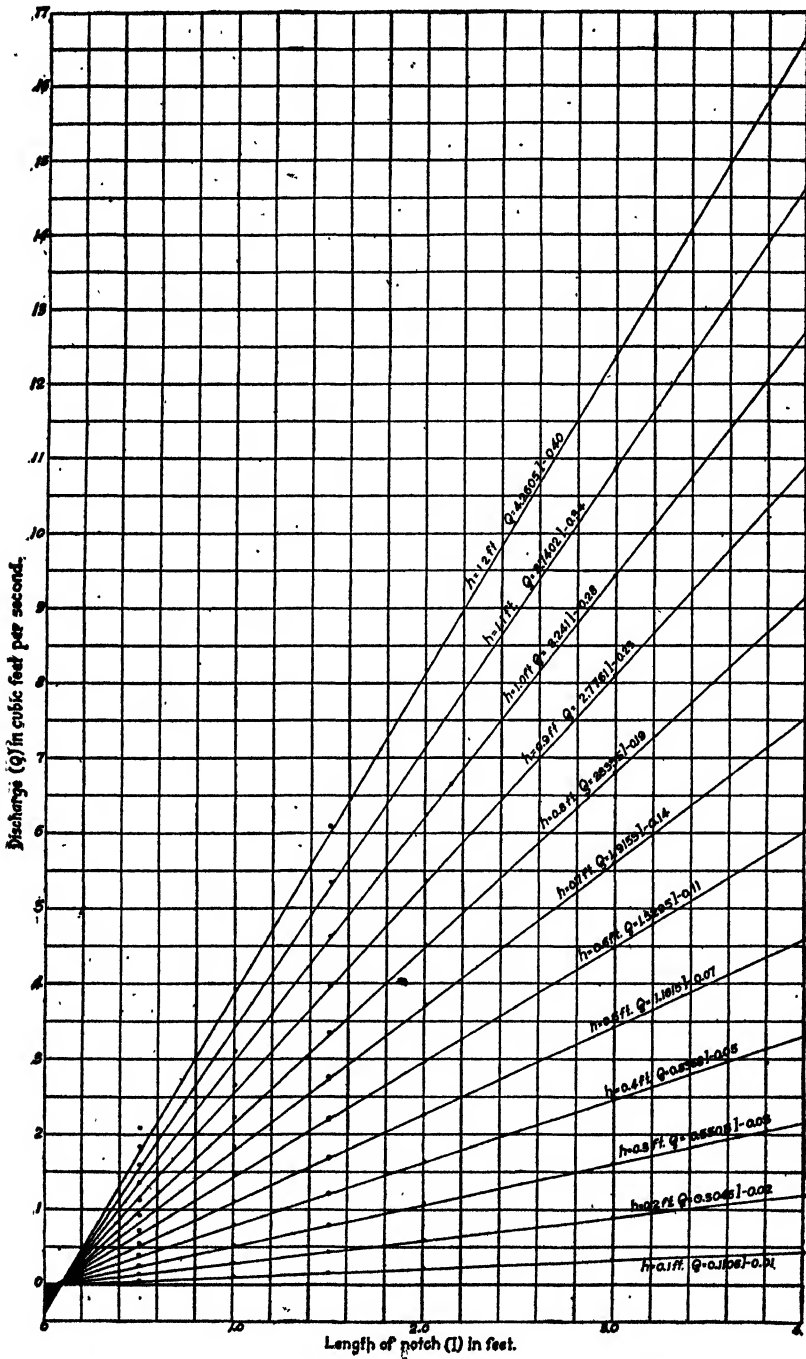


FIG. 4.—Curves showing the relation between discharges with constant heads through rectangular notches of different lengths and the lengths of the notches.

## RECTANGULAR NOTCHES

With rectangular notches 226 tests were made, the actual crest lengths used being 0.50721 foot, 1.0055 feet, 1.5026 feet, 2.0057 feet, 2.9970 feet, and 4.0065 feet. These actual lengths were used in all computations connected with the derivation of the formula.

## DERIVATION OF THE FORMULA

The discharge values for 0.05-foot increments of head, taken from the curves plotted from the experimental data, were used in the following deductions, thereby eliminating to a large extent the experimental

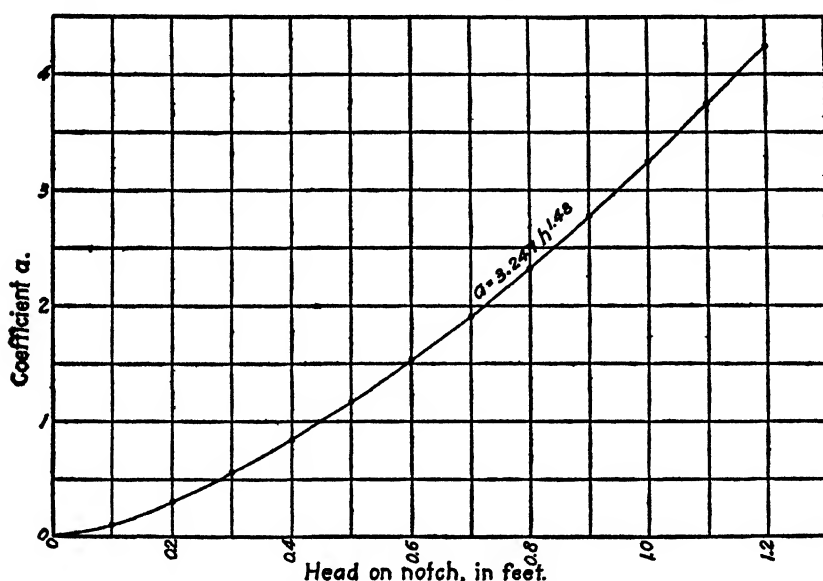


FIG. 5.—Curve showing the relation between  $a$  in the equation  $Q=aL-b$  and the heads on rectangular notches.

irregularities. The discharge values for the different notches were plotted (fig. 4) with the lengths of crests ( $L$ ) as abscissas, and the discharges ( $Q$ ) as ordinates. A straight line was then drawn for each head by passing it through the points representing the discharges over the 3- and 4-foot crests with the given head. The equations of these straight lines were found to be of the form  $Q=aL-b$ .

The slopes ( $a$ ) of the lines were computed from the coordinates of the discharge values with the 3- and 4-foot crests. The relations between the heads ( $H$ ) and the slopes ( $a$ ) in the above formula were plotted (fig. 5) and gave a curve the equation for which was found to be  $a=3.247H^{1.48}$ .

The relations between the heads ( $H$ ) and the intercepts ( $b$ ) in the equation  $Q=aL-b$  are shown in figure 6. The equation for the curve was found to be  $b=0.283H^{1.2}$ .



The offsets from each of the straight lines in figure 4 to the points representing the discharges with the head for which the line was drawn were tabulated, and an expression for the offsets was determined to be  $\frac{0.283H^{1.9}}{1+2L^{1.8}}$ .

Substituting the values of  $a$  and  $b$  in the equation form  $Q=aL-b$  and making a correction for the offsets from the straight lines, the formula for the rectangular notches was found to be

$$Q=3.247 LH^{1.48}-\left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9}$$

Table II gives the discharge values for the rectangular notches of different lengths computed by this formula. This formula gives discharge

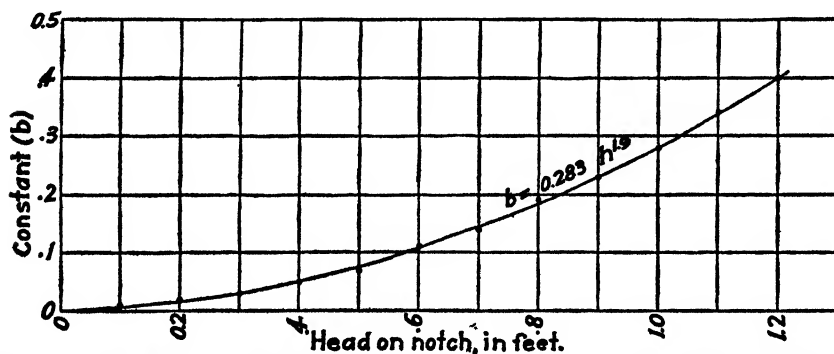


FIG. 6.—Curve showing the relation between  $b$  in the equation  $Q=aL-b$  and the heads on rectangular notches.

values within a maximum of approximately 1.2 per cent of the values indicated on the curves plotted from the experimental data, but the average variation is only 0.28 per cent. Table V compares the values indicated on the curves plotted from the experimental data and values computed with formulas.

TABLE II.—Discharges (in cubic feet per second) through rectangular weir notches <sup>1</sup>

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
0.20	2 3/8	0.291	0.439	0.588	0.887	1.19
.21	2 7/8	.312	.472	.632	.954	1.28
.22	2 5/8	.335	.505	.677	1.02	1.37
.23	2 3/4	.358	.539	.723	1.09	1.46
.24	2 1/8	.380	.574	.769	1.16	1.55

<sup>1</sup> Computed by the formula  $Q=3.247LH^{1.48}-\left(\frac{0.566L^{1.8}}{1+2L^{1.8}}\right)H^{1.9}$

TABLE II.—Discharges (in cubic feet per second) through rectangular weir notches—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
0.25	3	0.404	0.609	0.817	1.23	1.65
.26	3 $\frac{1}{8}$	.428	.646	.865	1.31	1.75
.27	3 $\frac{1}{4}$	.452	.682	.914	1.38	1.85
.28	3 $\frac{3}{8}$	.477	.720	.965	1.46	1.95
.29	3 $\frac{1}{2}$	.502	.758	1.02	1.53	2.05
.30	3 $\frac{5}{8}$	.527	.796	1.07	1.61	2.16
.31	3 $\frac{3}{4}$	.553	.836	1.12	1.69	2.27
.32	3 $\frac{7}{8}$	.580	.876	1.18	1.77	2.37
.33	4	.606	.916	1.23	1.86	2.48
.34	4 $\frac{1}{8}$	.634	.957	1.28	1.94	2.60
.35	4 $\frac{1}{4}$	.661	.999	1.34	2.02	2.71
.36	4 $\frac{3}{8}$	.688	1.04	1.40	2.11	2.82
.37	4 $\frac{1}{2}$	.717	1.08	1.45	2.20	2.94
.38	4 $\frac{3}{4}$	.745	1.13	1.51	2.28	3.06
.39	4 $\frac{7}{8}$	.774	1.17	1.57	2.37	3.18
.40	5	.804	1.21	1.63	2.46	3.30
.41	5 $\frac{1}{8}$	.833	1.26	1.69	2.55	3.42
.42	5 $\frac{1}{4}$	.863	1.30	1.75	2.65	3.54
.43	5 $\frac{3}{8}$	.893	1.35	1.81	2.74	3.67
.44	5 $\frac{1}{2}$	.924	1.40	1.88	2.83	3.80
.45	5 $\frac{3}{4}$	.955	1.44	1.94	2.93	3.93
.46	5 $\frac{7}{8}$	.986	1.49	2.00	3.03	4.05
.47	6	1.02	1.54	2.07	3.12	4.18
.48	6 $\frac{1}{8}$	1.05	1.59	2.13	3.22	4.32
.49	6 $\frac{1}{4}$	1.08	1.64	2.20	3.32	4.45
.50	6 $\frac{3}{8}$	1.11	1.68	2.26	3.42	4.58
.51	6 $\frac{1}{2}$	1.15	1.73	2.33	3.52	4.72
.52	6 $\frac{3}{4}$	1.18	1.78	2.40	3.62	4.86
.53	6 $\frac{7}{8}$	1.21	1.84	2.46	3.73	4.99
.54	7	1.25	1.89	2.53	3.83	5.13
.55	7 $\frac{1}{8}$	1.28	1.94	2.60	3.94	5.27
.56	7 $\frac{1}{4}$	1.31	1.99	2.67	4.04	5.42
.57	7 $\frac{3}{8}$	1.35	2.04	2.74	4.15	5.56
.58	7 $\frac{1}{2}$	1.38	2.09	2.81	4.26	5.70
.59	7 $\frac{3}{4}$	1.42	2.15	2.88	4.36	5.85
.60	7 $\frac{7}{8}$	1.45	2.20	2.96	4.47	6.00
.61	8	1.49	2.25	3.03	4.58	6.14
.62	8 $\frac{1}{8}$	1.52	2.31	3.10	4.69	6.29
.63	8 $\frac{1}{4}$	1.56	2.36	3.17	4.81	6.44
.64	8 $\frac{3}{8}$	1.60	2.42	3.25	4.92	6.59
.65	8 $\frac{1}{2}$	1.63	2.47	3.33	5.03	6.75
.66	8 $\frac{3}{4}$	1.67	2.53	3.40	5.15	6.90
.67	8 $\frac{7}{8}$	1.71	2.59	3.48	5.26	7.05
.68	9	1.74	2.64	3.56	5.38	7.21
.69	9 $\frac{1}{8}$	1.78	2.70	3.63	5.49	7.36
.70	9 $\frac{1}{4}$	1.82	2.76	3.71	5.61	7.52
.71	9 $\frac{3}{8}$	1.86	2.81	3.78	5.73	7.68
.72	9 $\frac{1}{2}$	1.90	2.87	3.86	5.85	7.84
.73	9 $\frac{3}{4}$	1.93	2.93	3.94	5.97	8.00
.74	9 $\frac{7}{8}$	1.97	2.99	4.02	6.09	8.17

TABLE II.—Discharges (in cubic feet per second) through rectangular weir notches—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
0.75	9	2.01	3.05	4.10	6.21	8.33
.76	9½	2.05	3.11	4.18	6.33	8.49
.77	9¾	2.09	3.17	4.26	6.45	8.66
.78	9⅝	2.13	3.23	4.34	6.58	8.82
.79	9½	2.17	3.29	4.42	6.70	8.99
.80	9⅝	2.21	3.35	4.51	6.83	9.16
.81	9¾	2.25	3.41	4.59	6.95	9.33
.82	9⅞	2.29	3.47	4.67	7.08	9.50
.83	9⅞	2.33	3.54	4.75	7.21	9.67
.84	10⅛	2.37	3.60	4.84	7.33	9.84
.85	10⅛	2.41	3.66	4.92	7.46	10.01
.86	10⅛	2.46	3.72	5.01	7.59	10.19
.87	10⅞	2.50	3.79	5.10	7.72	10.36
.88	10⅞	2.54	3.85	5.18	7.85	10.54
.89	10⅞	2.58	3.92	5.27	7.99	10.71
.90	10⅞	2.62	3.98	5.35	8.12	10.89
.91	10⅞	2.67	4.05	5.44	8.25	11.07
.92	11⅛	2.71	4.11	5.53	8.38	11.25
.93	11⅛	2.75	4.18	5.62	8.52	11.43
.94	11¼	2.79	4.24	5.71	8.65	11.61
.95	11¾	2.84	4.31	5.80	8.79	11.79
.96	11½	2.88	4.37	5.89	8.93	11.98
.97	11¾	2.93	4.44	5.98	9.06	12.16
.98	11¾	2.97	4.51	6.07	9.20	12.34
.99	11½	3.01	4.57	6.15	9.34	12.53
1.00	12	3.06	4.64	6.25	9.48	12.72
1.01	12½	.....	4.71	6.34	9.62	12.91
1.02	12¼	.....	4.78	6.43	9.76	13.10
1.03	12¾	.....	4.85	6.52	9.90	13.28
1.04	12½	.....	4.92	6.62	10.04	13.47
1.05	12¾	.....	4.98	6.71	10.18	13.66
1.06	12¾	.....	5.05	6.80	10.32	13.85
1.07	12⅞	.....	5.12	6.90	10.46	14.04
1.08	12⅞	.....	5.19	6.99	10.61	14.24
1.09	13⅛	.....	5.26	7.09	10.75	14.43
1.10	13⅛	.....	5.34	7.19	10.90	14.64
1.11	13⅛	.....	5.41	7.28	11.04	14.83
1.12	13⅞	.....	5.48	7.38	11.19	15.03
1.13	13⅞	.....	5.55	7.47	11.34	15.22
1.14	13⅞	.....	5.62	7.57	11.49	15.42
1.15	13⅞	.....	5.69	7.66	11.64	15.62
1.16	13⅞	.....	5.77	7.76	11.79	15.82
1.17	14⅛	.....	5.84	7.86	11.94	16.02
1.18	14⅛	.....	5.91	7.96	12.09	16.23
1.19	14¼	.....	5.98	8.06	12.24	16.43
1.20	14¾	.....	6.06	8.16	12.39	16.63
1.21	14½	.....	6.13	8.26	12.54	16.83
1.22	14¾	.....	6.20	8.36	12.69	17.04
1.23	14¾	.....	6.28	8.46	12.85	17.25
1.24	14¾	.....	6.35	8.56	12.99	17.45

TABLE II.—Discharges (in cubic feet per second) through rectangular weir notches—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
1. 25	15		6. 43	8. 66	13. 14	17. 66
1. 26	15½				13. 30	17. 87
1. 27	15¾				13. 45	18. 07
1. 28	15¾				13. 61	18. 28
1. 29	15½				13. 77	18. 50
1. 30	15½				13. 93	18. 71
1. 31	15¾				14. 09	18. 92
1. 32	15¾				14. 24	19. 13
1. 33	15¾				14. 40	19. 34
1. 34	16¼				14. 56	19. 55
1. 35	16¼				14. 72	19. 77
1. 36	16¼				14. 88	19. 98
1. 37	16¼				15. 04	20. 20
1. 38	16¼				15. 20	20. 42
1. 39	16¼				15. 36	20. 64
1. 40	16¼				15. 53	20. 86
1. 41	16¼				15. 69	21. 08
1. 42	17¼				15. 85	21. 30
1. 43	17¼				16. 02	21. 52
1. 44	17¼				16. 19	21. 74
1. 45	17¾				16. 34	21. 96
1. 46	17¾				16. 51	22. 18
1. 47	17¾				16. 68	22. 41
1. 48	17¾				16. 85	22. 63
1. 49	17¾				17. 01	22. 85
1. 50	18				17. 18	23. 08

The discharges through a notch having a crest length of 0.5 foot did not follow the same law as those through larger notches. This was probably owing to the greater effect of friction in the smaller notch and to the interference due to the end-contraction filaments of flow crossing each other in the middle of the notch section. The formula

$$Q = 1.593H^{1.526} \left( 1 + \frac{1}{800H^{2.3}} \right)$$

was found to give discharge values consistent with the curve plotted from experimental data for the 0.5-foot notch. The use of such a notch is very limited, and the 90° triangular notch is as accurate and much more satisfactory.

#### COMPARISON OF THE FRANCIS FORMULA AND THE NEW FORMULA

The discharge values obtained for rectangular notches by the Francis and the new formulas are shown in graphic form in figure 7 and in tabular form in Table III.

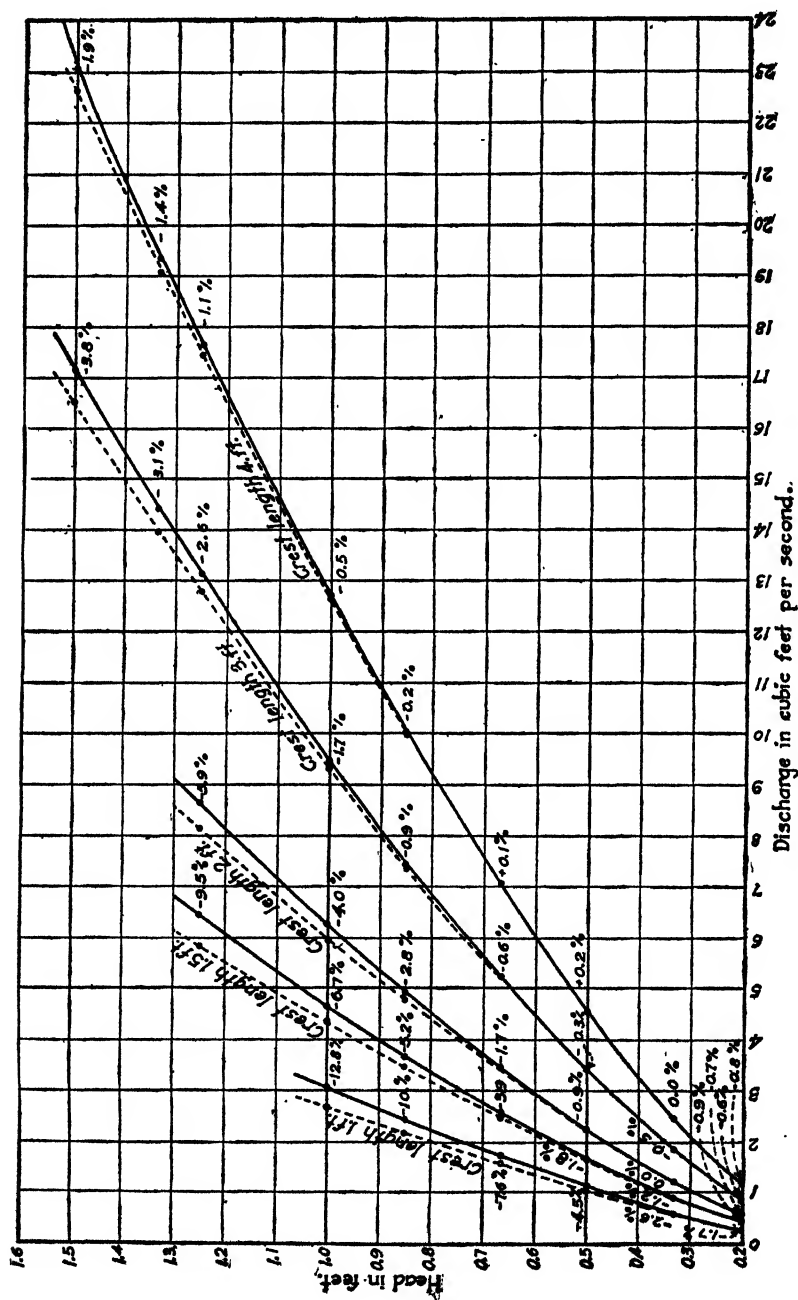


FIG. 7.—Curves showing discharges through rectangular notches of different lengths.

TABLE III.—Comparison of discharges through rectangular notches computed from the Francis formula and the new formula

Head.  <i>Feet.</i>	1-foot crest.			1½-foot crest.			2-foot crest.			3-foot crest.			4-foot crest.		
	Discharge computed by the Francis formula. <sup>1</sup>		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula. <sup>1</sup>		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula. <sup>1</sup>		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula. <sup>1</sup>		Discharge computed by new formula (cubic feet per second).	Discharge computed by the Francis formula. <sup>1</sup>		Discharge computed by new formula (cubic feet per second).
	Amount	Percentage of discharge computed by new formula.		Amount	Percentage of discharge computed by new formula.		Amount	Percentage of discharge computed by new formula.		Amount	Percentage of discharge computed by new formula.		Amount	Percentage of discharge computed by new formula.	
0.20	0.291	98.3	0.286	0.435	99.1	0.588	0.584	99.3	0.887	0.882	99.4	1.19	1.18	99.2	
0.33	0.606	97.4	0.590	0.905	98.8	1.22	1.22	100.0	1.86	1.85	99.5	2.48	2.48	100.0	
0.50	1.12	95.5	1.06	1.65	98.2	2.26	2.24	99.1	3.42	3.41	99.7	4.58	4.59	100.2	
0.67	1.71	92.4	1.58	2.49	96.1	3.47	3.41	98.3	5.26	5.23	99.4	7.05	7.06	100.1	
0.85	2.41	90.0	2.17	3.47	94.8	4.92	4.78	97.2	7.45	7.38	99.1	10.01	9.99	99.8	
1.00	3.06	87.2	2.67	4.33	93.3	6.24	5.99	96.0	9.48	9.32	98.3	12.72	12.65	99.5	
1.25	.....	.....	.....	5.82	90.5	8.65	8.14	94.1	13.14	12.80	97.4	17.65	17.45	98.9	
1.33	.....	.....	.....	6.43	.....	.....	.....	.....	14.40	13.96	98.9	19.34	19.07	98.6	
1.50	.....	.....	.....	.....	.....	.....	.....	.....	17.17	16.52	98.2	23.08	22.64	98.1	

<sup>1</sup> The Francis formula:  $Q = 3.33(L - 0.2H)H^{3/2}$ .

The curves and Table III show that except for a small range of heads on the 4-foot notch the discharges computed by the Francis formula are too small. The actual discharges, however, where the head did not exceed one-third of the length of the crest, did not vary much from those computed by the Francis formula and support the statement of Francis that his formula would give discharge values correct to within 2 per cent, provided the head does not exceed one-third the length of the crest. Nevertheless the fact that the curves plotted from the experimental data have no sudden breaks or changes of direction shows that no limit need be placed upon the head, provided the proper formula is used to compute the discharge. It also shows that the necessity of the limit on the application of the Francis formula was due to the mathematical shortcoming of the formula and not to any peculiarity inherent in the rectangular notch. The new formula not only gives greater accuracy within the range of the Francis formula but also permits the accurate measurement of discharges with the heads exceeding one-third the length of the crest. The maximum limit of the ratio of the head to the crest length with the new formula has not been ascertained, the greatest ratio experimented with being 1 to 1 with the 1-foot notch. The parts of all the curves showing the discharges with higher heads, however, were quite consistent in all cases with the rest of the curves. A head of 1 foot was run over a 0.5-foot notch, but the results were inconclusive, as the discharges through the 0.5-foot notch do not follow the general formula.

The new formula is more complicated than the Francis formula, but gives discharge values which are more accurate within the limits of these experiments, and since tables are generally consulted to determine the flow that is passing through a notch, the practical disadvantage of the new formula is largely overcome. If one is obliged to use a formula in the field for computing the discharge, an approximation usually is sufficient, and the Francis formula gives discharges sufficiently accurate for practical needs.

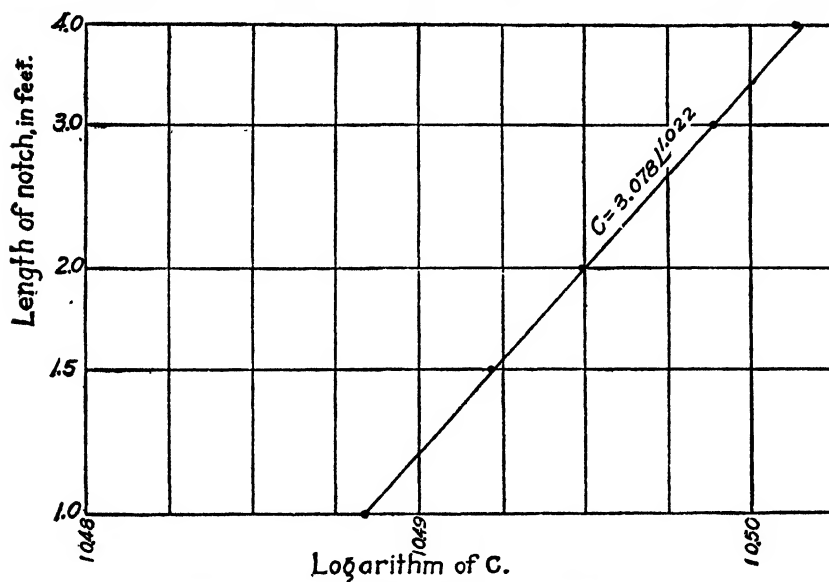
#### STRAIGHT-LINE FORMULA

As stated on page 1059, it was found, when the experimental data for the rectangular notches were plotted logarithmically, that a general straight-line formula could be deduced which, within the range of the experiments, would give discharge values as close to the plotted values as did the general formula deduced above. The equations for the straight lines best representing the discharges with the given heads through the different notches were found to be as shown in Table IV.

TABLE IV.—Equations for straight lines representing discharges through rectangular weir notches

Length of crest.	Equations of line.
<i>Feet.</i>	
1. 0055	$Q=3.078LH^{1.463}$
1. 5026	$Q=3.106LH^{1.465}$
2. 0057	$Q=3.125LH^{1.466}$
2. 9970	$Q=3.154LH^{1.467}$
4. 0056	$Q=3.172LH^{1.473}$

The coefficient values ( $C$ ) in the above equations were plotted (fig. 8) against the lengths of crests ( $L$ ), and the exponent values

FIG. 8.—Curve showing relation of coefficients ( $C$ ) to lengths of rectangular notches.

( $n$ ) were plotted (fig. 9) against the lengths of crests ( $L$ ). Average straight lines drawn to represent the points were found to have the equations  $C = 3.078L^{1.022}$  and  $n = 1.46 + 0.003L$ .

Substituting these values of  $C$  and  $n$  in the equation  $Q = CLH^n$ , the formula for the discharge through rectangular notches was found to be

$$Q = 3.08L^{1.022}H^{(1.46+.003L)}.$$

This formula gives discharge values that agree within a maximum of 0.7 per cent with the values indicated on the curves plotted from the experimental data, but the average variation is only 0.26 per cent.

Table V gives the discharges through the notches used, computed by the curve and by the straight-line formulas, also the values indicated on the curves plotted from the experimental data.



TABLE V.—Discharges (in cubic feet per second) for rectangular notches as shown by curves plotted from experimental data, and discharges computed by curve and straight-line formulas

Head.	1.0055-foot notch.			1.5026-foot notch.			2.0057-foot notch.			2.997-foot notch.			4.0056-foot notch.		
	Experimental data.	Curve formula.	Straight - line formula.	Experimental data.	Curve formula.	Straight - line formula.	Experimental data.	Curve formula.	Straight - line formula.	Experimental data.	Curve formula.	Straight - line formula.	Experimental data.	Curve formula.	Straight - line formula.
Feet.															
0.2	0.293	0.293	0.294	0.443	0.440	0.442	0.593	0.590	0.593	0.890	0.886	0.889	1.194	1.189	1.190
0.3	.531	.530	.532	.800	.797	.801	1.079	1.071	1.074	1.617	1.610	1.613	2.163	2.161	2.162
0.4	.806	.808	.811	1.220	1.217	1.220	1.640	1.635	1.637	2.461	2.462	2.461	3.302	3.304	3.302
0.5	1.115	1.120	1.123	1.680	1.688	1.692	2.267	2.268	2.271	3.411	3.418	3.416	4.594	4.589	4.585
0.6	1.439	1.462	1.467	2.195	2.205	2.210	2.969	2.964	2.966	4.474	4.470	4.465	6.013	6.004	5.997
0.7	1.834	1.830	1.838	2.755	2.761	2.770	3.718	3.716	3.719	5.595	5.605	5.600	7.532	7.533	7.524
0.8	2.233	2.223	2.235	3.354	3.357	3.368	4.519	4.519	4.523	6.795	6.821	6.814	9.157	9.171	9.156
0.9	2.660	2.639	2.655	3.988	3.987	4.002	5.367	5.369	5.375	8.090	8.120	8.101	10.910	10.906	10.892
1.0	3.103	3.076	3.097	4.664	4.650	4.670	6.238	6.265	6.273	9.432	9.467	9.457	12.706	12.734	12.720
1.1	.....	.....	.....	5.370	5.346	5.369	7.190	7.205	7.214	10.866	10.893	10.878	14.642	14.656	14.635
1.2	.....	.....	.....	6.133	6.068	6.099	8.174	8.181	8.195	12.356	12.374	12.361	16.666	16.653	16.635
1.3	.....	.....	.....	6.903	6.819	6.857	9.193	9.196	9.215	13.876	13.918	13.903	.....	.....	.....

In locating the straight lines on the logarithmic plot, it was found that the points for the 1.0055-foot notch could be covered quite closely by three straight lines approximately equal in length. The same was approximately true of the points for the 1.5026-foot notch. Only two

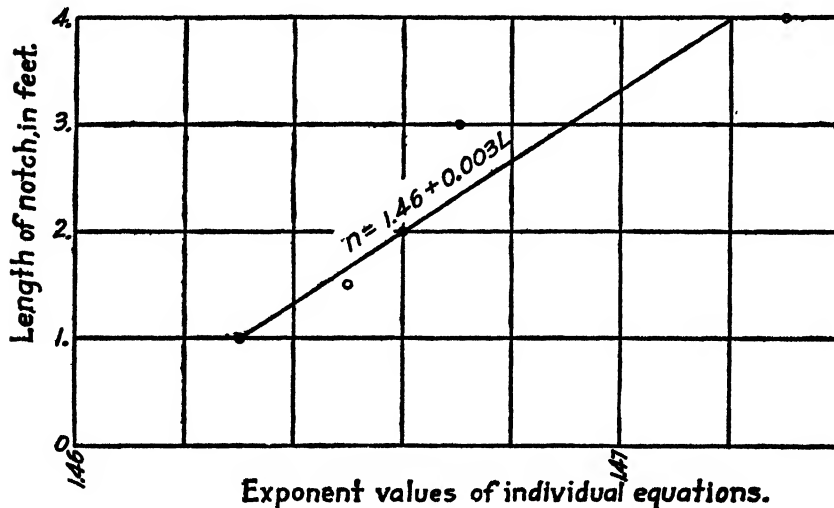


FIG. 9.—Curve showing relation of  $n$  to length of rectangular notches.

straight lines each, however, were required for the 2.0057-foot and 2.997-foot notches, although a third could be assumed near the upper part of the curves in each case. For the 4.0056-foot notch there was only one point of change, and it was well above the middle of the curve. These facts indicate that had large enough heads been run on the longer notches to give the same ratio of length of crest to head as was obtained with the

1-foot notch, an equal number of lines would have been required to cover the points. If a single straight line is taken to represent the discharge curve, and it is placed to represent best the discharges with the lower heads, as was done above, the part of the true discharge curve for the higher heads diverges rapidly from the straight line. The curve formula takes account of the law of variation of the discharge curves better than does the straight-line formula, and, consequently, it appears that it will give closer values for the higher heads and for longer notches than those experimented with.

The straight-line equation for the 0.5-foot notch was found to be  $Q = 1.566H^{1.504}$ .

This equation was found to give discharge values within approximately 1 per cent of the values indicated on the curve plotted from the experimental data.

#### CIPOLLETTI NOTCHES

With notches having side slopes of one horizontal to four vertical, 219 tests were made. The actual crest lengths used were 0.50062 foot, 1.0050 foot, 1.5028 feet, 2.0002 feet, 3.0011 feet, and 4.0058 feet, respectively, and these lengths were used throughout the following calculations.

#### DERIVATION OF THE FORMULA

The difference between the areas of a Cipolletti and a rectangular notch with equal crest length is the area of a  $28^{\circ} 4'$  (approximately) triangular notch—that is, one having one to four side slopes. It was found, however, that the discharges through such a notch (see Table X) with a given head did not exactly equal the difference between the discharges through a rectangular and a Cipolletti notch with equal crest lengths and the same head. While the differences between the discharges through the Cipolletti and rectangular notches increase with the head for all crest lengths, there was no regular increase or decrease in the differences in the discharges with increases in the crest lengths so long as the heads were less than approximately 0.8 foot, but for higher heads the differences in discharges decreased as the crest lengths increased. The comparison of the differences is very unreliable for heads as low as 0.2 or 0.3 foot. The discharges through the  $28^{\circ} 4'$  notch are greater than the differences between the discharges of the Cipolletti and rectangular notches for all heads up to approximately 2.5 feet, the percentages of excess decreasing with the increases in head and equaling zero with a head of approximately 2.5.

The differences between the discharges through the rectangular and Cipolletti notches for each of the crest lengths were determined from the curves plotted from the experimental data and an average made for each 0.1 foot of head. These averages were then plotted logarithmically against the head, and the equation of the curve representing the differ-

ence in discharge was found to be  $D = .609H^{2.5}$ . By adding the term  $.609H^{2.5}$  to the general formula for discharges through rectangular notches (page 1064), the general formula for discharges through Cipolletti notches was found to be

$$Q = 3.247LH^{1.48} - \left( \frac{0.566L^{1.5}}{1 + 2L^{1.5}} \right) H^{1.9} + 0.609H^{2.5}$$

This formula gives discharge values for 1-, 1½-, 2-, 3-, and 4-foot notches that agree within 0.5 per cent of the values indicated on the curves plotted from the experimental data, except for the lower heads on the 1-foot notch, where the maximum discrepancy, owing to the small discharge, is approximately 1½ per cent. The discrepancies are positive in some cases and negative in others. (See Table VII for discharge values indicated by the curves plotted from the experimental data and discharge values computed by the formulas.)

Table VI gives the discharge values for Cipolletti notches of different lengths computed by the new formula.

TABLE VI.—Discharges (in cubic feet per second) through Cipolletti weir notches<sup>1</sup>

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
0.20	2½	0.30	0.45	0.60	0.90	1.20
.21	2½	.32	.48	.64	.97	1.29
.22	2½	.35	.52	.69	1.04	1.38
.23	2¾	.37	.55	.74	1.11	1.47
.24	2¾	.39	.59	.79	1.18	1.57
.25	3	.42	.63	.84	1.25	1.67
.26	3½	.45	.67	.89	1.33	1.77
.27	3½	.47	.71	.94	1.40	1.87
.28	3½	.50	.75	.99	1.48	1.97
.29	3½	.53	.79	1.04	1.56	2.08
.30	3½	.56	.83	1.10	1.64	2.19
.31	3¾	.59	.87	1.15	1.73	2.30
.32	3¾	.61	.91	1.21	1.81	2.41
.33	3¾	.64	.95	1.27	1.89	2.52
.34	4½	.67	1.00	1.32	1.98	2.64
.35	4½	.70	1.04	1.38	2.07	2.75
.36	4½	.73	1.09	1.44	2.16	2.87
.37	4½	.77	1.13	1.50	2.25	2.99
.38	4½	.80	1.18	1.57	2.34	3.11
.39	4½	.83	1.23	1.63	2.43	3.24
.40	4½	.87	1.28	1.69	2.53	3.36
.41	4½	.90	1.32	1.76	2.62	3.49
.42	5½	.93	1.37	1.82	2.72	3.61
.43	5½	.97	1.42	1.89	2.81	3.74
.44	5½	1.00	1.47	1.95	2.91	3.87

<sup>1</sup> Computed by the formula  $Q = 3.247LH^{1.48} - \left( \frac{0.566L^{1.5}}{1 + 2L^{1.5}} \right) H^{1.9} + 0.609H^{2.5}$

TABLE VI.—Discharges (in cubic feet per second) through Cipolletti weir notches—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
<i>Feet.</i>	<i>Inches.</i>					
0.45	5½	1.04	1.53	2.02	3.01	4.01
.46	5½	1.07	1.58	2.09	3.11	4.14
.47	5½	1.11	1.63	2.16	3.21	4.28
.48	5¾	1.15	1.68	2.23	3.32	4.41
.49	5¾	1.18	1.74	2.30	3.42	4.55
.50	6	1.22	1.79	2.37	3.53	4.69
.51	6½	1.26	1.85	2.44	3.64	4.83
.52	6½	1.30	1.90	2.51	3.74	4.97
.53	6¾	1.34	1.96	2.59	3.85	5.12
.54	6¾	1.38	2.02	2.66	3.96	5.26
.55	6¾	1.42	2.07	2.74	4.07	5.41
.56	6¾	1.46	2.13	2.81	4.18	5.56
.57	6¾	1.50	2.19	2.89	4.30	5.71
.58	6¾	1.54	2.25	2.97	4.41	5.86
.59	7	1.58	2.31	3.05	4.53	6.01
.60	7	1.62	2.37	3.13	4.64	6.17
.61	7	1.67	2.43	3.20	4.76	6.32
.62	7	1.71	2.49	3.28	4.88	6.47
.63	7	1.75	2.55	3.37	5.00	6.63
.64	7	1.80	2.62	3.45	5.12	6.79
.65	7	1.84	2.68	3.53	5.24	6.95
.66	7	1.89	2.75	3.61	5.36	7.11
.67	7	1.93	2.81	3.70	5.48	7.28
.68	7	1.98	2.87	3.79	5.61	7.44
.69	7	2.02	2.94	3.87	5.73	7.61
.70	8	2.07	3.01	3.95	5.86	7.77
.71	8	2.12	3.07	4.04	5.98	7.94
.72	8	2.16	3.14	4.13	6.11	8.11
.73	8	2.21	3.21	4.22	6.24	8.28
.74	8	2.26	3.28	4.31	6.38	8.45
.75	9	2.31	3.35	4.40	6.51	8.62
.76	9	2.36	3.42	4.49	6.64	8.80
.77	9	2.41	3.49	4.58	6.77	8.97
.78	9	2.46	3.56	4.67	6.90	9.15
.79	9	2.51	3.63	4.76	7.04	9.33
.80	9	2.56	3.70	4.85	7.18	9.51
.81	9	2.61	3.77	4.95	7.31	9.69
.82	9	2.66	3.84	5.04	7.45	9.87
.83	9	2.71	3.92	5.14	7.59	10.05
.84	10	2.77	3.99	5.23	7.73	10.23
.85	10	2.82	4.07	5.33	7.87	10.42
.86	10	2.87	4.14	5.43	8.01	10.60
.87	10	2.93	4.22	5.52	8.15	10.79
.88	10	2.98	4.29	5.62	8.30	10.98
.89	10	3.04	4.37	5.72	8.44	11.17

TABLE VI.—Discharges (in cubic feet per second) through Cipolletti weir notches—*Con.*

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
Feet.	Inches.					
0.90	10 $\frac{1}{8}$	3.09	4.45	5.82	8.59	11.36
.91	10 $\frac{1}{4}$	3.15	4.53	5.92	8.73	11.55
.92	11 $\frac{1}{8}$	3.20	4.60	6.02	8.88	11.74
.93	11 $\frac{1}{4}$	3.26	4.68	6.13	9.03	11.94
.94	11 $\frac{3}{8}$	3.32	4.76	6.23	9.17	12.13
.95	11 $\frac{1}{2}$	3.37	4.84	6.33	9.32	12.33
.96	11 $\frac{3}{4}$	3.43	4.92	6.44	9.47	12.53
.97	12 $\frac{1}{8}$	3.49	5.00	6.55	9.62	12.72
.98	12 $\frac{1}{4}$	3.55	5.09	6.64	9.78	12.92
.99	12 $\frac{3}{8}$	3.61	5.17	6.75	9.93	13.12
1.00	12	3.67	5.25	6.86	10.08	13.32
1.01	12 $\frac{1}{8}$	.....	5.33	6.96	10.24	13.53
1.02	12 $\frac{1}{4}$	.....	5.42	7.07	10.40	13.73
1.03	12 $\frac{3}{8}$	.....	5.50	7.18	10.55	13.94
1.04	12 $\frac{1}{2}$	.....	5.59	7.29	10.71	14.15
1.05	12 $\frac{3}{4}$	.....	5.67	7.40	10.87	14.35
1.06	13 $\frac{1}{8}$	.....	5.76	7.51	11.03	14.56
1.07	13 $\frac{1}{4}$	.....	5.84	7.62	11.19	14.77
1.08	13 $\frac{3}{8}$	.....	5.93	7.73	11.35	14.98
1.09	13 $\frac{1}{2}$	.....	6.02	7.84	11.51	15.19
1.10	13 $\frac{3}{4}$	.....	6.11	7.96	11.68	15.41
1.11	14 $\frac{1}{8}$	.....	6.20	8.07	11.84	15.62
1.12	14 $\frac{1}{4}$	.....	6.29	8.18	12.00	15.83
1.13	14 $\frac{3}{8}$	.....	6.38	8.29	12.16	16.04
1.14	14 $\frac{1}{2}$	.....	6.47	8.41	12.33	16.26
1.15	14 $\frac{3}{4}$	.....	6.56	8.53	12.50	16.48
1.16	15 $\frac{1}{8}$	.....	6.65	8.65	12.67	16.70
1.17	15 $\frac{1}{4}$	.....	6.74	8.76	12.84	16.93
1.18	15 $\frac{3}{8}$	.....	6.83	8.88	13.01	17.15
1.19	15 $\frac{1}{2}$	.....	6.93	9.00	13.18	17.37
1.20	15 $\frac{3}{4}$	.....	7.02	9.12	13.35	17.59
1.21	16 $\frac{1}{8}$	.....	7.11	9.24	13.52	17.81
1.22	16 $\frac{1}{4}$	.....	7.20	9.36	13.69	18.03
1.23	16 $\frac{3}{8}$	.....	7.30	9.48	13.87	18.26
1.24	16 $\frac{1}{2}$	.....	7.40	9.60	14.04	18.49
1.25	15	.....	7.49	9.72	14.21	18.71
1.26	15 $\frac{1}{8}$	.....	.....	.....	14.39	18.94
1.27	15 $\frac{1}{4}$	.....	.....	.....	14.56	19.17
1.28	15 $\frac{3}{8}$	.....	.....	.....	14.74	19.41
1.29	15 $\frac{1}{2}$	.....	.....	.....	14.92	19.65
1.30	15 $\frac{3}{4}$	.....	.....	.....	15.11	19.88
1.31	16 $\frac{1}{8}$	.....	.....	.....	15.29	20.12
1.32	16 $\frac{1}{4}$	.....	.....	.....	15.46	20.35
1.33	16 $\frac{3}{8}$	.....	.....	.....	15.64	20.58
1.34	16 $\frac{1}{2}$	.....	.....	.....	15.82	20.82

TABLE VI.—Discharges (in cubic feet per second) through Cipolletti weir notches—Con.

Head.		1-foot crest.	1½-foot crest.	2-foot crest.	3-foot crest.	4-foot crest.
<i>Feet.</i>	<i>Inches.</i>					
1.35	16 $\frac{1}{8}$	.....	.....	.....	16.01	21.06
1.36	16 $\frac{1}{4}$	.....	.....	.....	16.19	21.29
1.37	16 $\frac{3}{8}$	.....	.....	.....	16.37	21.53
1.38	16 $\frac{1}{2}$	.....	.....	.....	16.56	21.78
1.39	16 $\frac{3}{4}$	.....	.....	.....	16.75	22.02
1.40	16 $\frac{7}{8}$	.....	.....	.....	16.94	22.27
1.41	17	.....	.....	.....	17.13	22.51
1.42	17 $\frac{1}{8}$	.....	.....	.....	17.32	22.75
1.43	17 $\frac{1}{4}$	.....	.....	.....	17.51	23.00
1.44	17 $\frac{3}{8}$	.....	.....	.....	17.70	23.25
1.45	17 $\frac{1}{2}$	.....	.....	.....	17.89	23.50
1.46	17 $\frac{3}{4}$	.....	.....	.....	18.08	23.75
1.47	17 $\frac{7}{8}$	.....	.....	.....	18.28	24.00
1.48	18	.....	.....	.....	18.47	24.25
1.49	18 $\frac{1}{8}$	.....	.....	.....	18.66	24.50
1.50	18 $\frac{1}{4}$	.....	.....	.....	18.85	24.75

The discharges through the Cipolletti notch, having a nominal crest length of 0.5 foot, did not follow the same law as those through the longer notches, possibly for the reasons noted on page 1067 for the 0.5-foot rectangular notch, and the use of such notches should be discouraged in favor of the 90° triangular notch, which measures small discharges more accurately.

The following formula represents the flow through the 0.5-foot Cipolletti notch, but is stated here only for technical reasons:

$$Q = 1.593H^{1.526} \left( 1 + \frac{1}{800H^{2.3}} \right) 0.587H^{2.53}$$

#### COMPARISON OF THE CIPOLLETTI FORMULA AND THE NEW FORMULA

The discharge values computed by the Cipolletti and new formulas are shown in graphic form in figure 10 and in tabular form in Table VII.

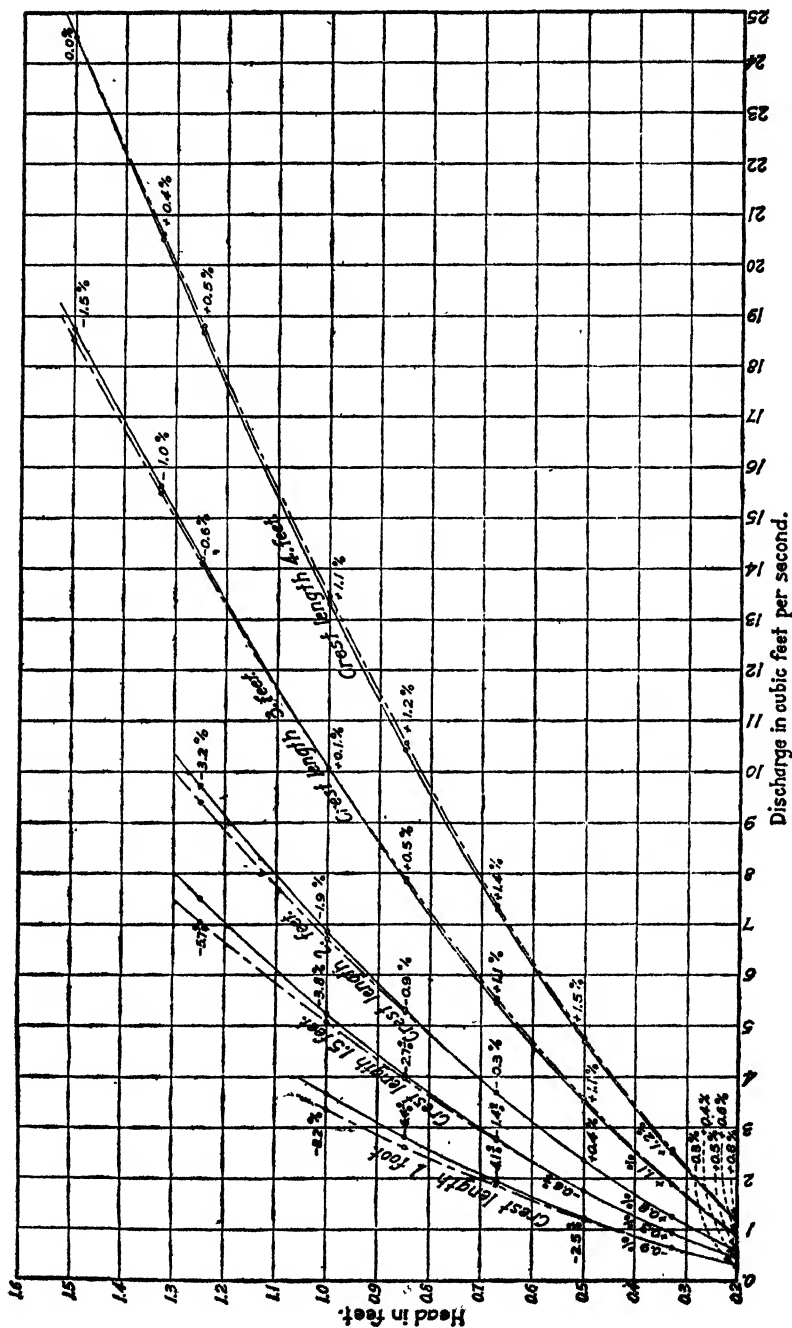


FIG. 10.—Curves showing discharges through Cipolletti weir notches of different lengths.

TABLE VII.—Comparison of discharges through trapezoidal notches with side slopes of 1:4 computed by the Cipolletti formula and by the new formula.<sup>1</sup>

Head.	1-foot crest.			1½-foot crest.			2-foot crest.			3-foot crest.			4-foot crest.		
	Discharge computed by new formula		Discharge computed by Cipolletti formula.	Discharge computed by new formula		Discharge computed by Cipolletti formula.	Discharge computed by new formula		Discharge computed by Cipolletti formula.	Discharge computed by new formula		Discharge computed by Cipolletti formula.	Discharge computed by new formula		Discharge computed by Cipolletti formula.
	Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.		Amount (cubic feet per second).	Percentage of discharge computed by new formula.	
<i>F</i> feet.	0.302	99.7	0.450	0.452	100.4	0.599	0.602	100.5	0.808	0.903	100.6	1.20	1.21	100.8	
0.33	0.644	99.1	0.954	0.957	100.3	1.27	1.28	100.8	1.89	1.91	101.1	2.52	2.55	101.2	
0.36	1.22	97.5	1.70	1.73	99.4	2.37	2.38	100.4	3.43	3.47	101.1	4.60	4.70	101.5	
0.67	1.93	95.9	2.81	2.77	98.6	3.76	3.69	99.7	5.48	5.54	101.1	7.38	7.38	101.4	
0.85	2.82	93.6	4.07	3.96	97.3	5.13	5.28	99.1	7.87	7.91	100.5	10.43	10.52	101.2	
1.00	3.67	91.8	5.23	5.05	96.2	6.86	6.73	98.5	10.09	10.10	100.7	13.31	13.47	101.1	
1.25	.....	.....	7.49	7.06	94.3	9.72	9.41	96.8	14.41	14.12	99.4	18.72	18.82	100.5	
1.33	.....	.....	.....	.....	.....	.....	.....	.....	15.84	15.49	99.0	20.18	20.66	100.4	
2.50	.....	.....	.....	.....	.....	.....	.....	.....	18.85	18.50	98.5	24.75	24.74	100.0	

<sup>1</sup> Cipolletti formula:  $Q = 3.367LH^{3/2}$



The curves and the table show that with heads less than one-third the length of the crest the Cipolletti formula gives discharge values within 1.5 per cent of the actual discharges, therefore being somewhat more accurate than the Francis formula. The new formula, like the new formula for the rectangular weir, is not only more nearly accurate than the old formula, but also permits the use of heads greater than one-third the crest length. The maximum limit of the ratio of the head to the crest length was not ascertained, but the parts of the curves for the higher heads are consistent, there being no sudden breaks or changes of direction.

The new formula is more complicated than the Cipolletti formula, but because of its greater degree of accuracy it should be used in computing tables. The Cipolletti formula, however, is sufficiently accurate for field computations where only approximate discharge values are required.

Cipolletti notches do not give discharges proportional to the lengths of the crest, as has been commonly claimed, and consequently notches of this type have no advantages over rectangular notches (see p. 1098).

#### FORMULA BASED ON THE STRAIGHT-LINE FORMULA FOR RECTANGULAR NOTCHES

The difference between the discharges computed by the new rectangular-notch formula and the discharges taken from the curves plotted from the experimental data for the Cipolletti notches were determined for each 0.1 foot of head for the several lengths of notches. These values were then plotted logarithmically against the heads, and the equation of the average straight line representing the difference in discharge was found to be  $D = .6H^{2.6}$ . By adding the term  $0.6H^{2.6}$  to the general formula for discharges through rectangular notches (p. 1071), the general formula for discharges through Cipolletti notches was found to be

$$Q = 3.08L^{1.022}H^{(1.46+0.008L)} + 0.6H^{2.6}$$

This formula gives discharge values that agree within a maximum of 1 per cent of the values indicated on the curves plotted from the experimental data, but the agreement is within 0.5 per cent for all but a very few points.

Table VIII gives the discharges through the notches used, computed by the two formulas deduced for the Cipolletti notches, and the discharge values indicated on the curves plotted from the experimental data.

TABLE VIII.—Discharges (in cubic feet per second) for Cipolletti weir notches as shown by curves plotted from experimental data, and discharges computed by formulas on pages 1074 and 1080

Head.	1.0050-foot notch.			1.5028-foot notch.			2.0002-foot notch.			3.0011-foot notch.			4.0058-foot notch.		
	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.	Experimental data.	According to formula on page 1074.	According to formula on page 1080.
<i>Feet.</i>															
0.2	0.300	0.302	0.303	0.455	0.450	0.451	0.600	0.600	0.602	0.902	0.900	0.900	0.898	1.206	1.200
0.3	.555	.563	.558	.829	.83	.827	1.109	1.10	1.100	1.647	1.64	1.64	1.639	2.193	2.19
0.4	.866	.874	.866	1.280	1.28	1.275	1.694	1.69	1.694	2.535	2.53	2.53	2.519	3.360	3.357
0.5	1.218	1.23	1.222	1.798	1.80	1.797	2.375	2.37	2.370	3.530	3.53	3.53	3.515	4.705	4.70
0.6	1.622	1.63	1.626	2.370	2.37	2.369	3.141	3.13	3.125	4.650	4.64	4.64	4.624	6.179	6.18
0.7	2.075	2.08	2.077	3.004	3.02	3.009	3.953	3.95	3.958	5.870	5.86	5.86	5.839	7.800	7.78
0.8	2.565	2.57	2.571	3.706	3.71	3.704	4.845	4.85	4.859	7.185	7.18	7.18	7.150	9.537	9.52
0.9	3.111	3.11	3.111	4.462	4.46	4.458	5.815	5.82	5.831	8.576	8.59	8.59	8.557	11.392	11.38
1.0	3.695	3.69	3.697	5.261	5.26	5.270	6.845	6.86	6.873	10.078	10.08	10.08	10.057	13.376	13.34
1.1	.....	.....	.....	6.137	6.12	6.138	7.941	7.96	7.983	11.655	11.68	11.68	11.647	15.425	15.43
1.2	.....	.....	.....	7.060	7.03	7.063	9.110	9.12	9.159	13.359	13.36	13.36	13.325	.....	.....

The differences between the discharges through the 0.5-foot Cipolletti notch obtained from the curves plotted from the experimental data and the discharges computed by the formula for the 0.5-foot rectangular notch were determined and plotted logarithmically against the heads. The straight line representing these differences has the equation  $D=0.56H^{2.55}$ . By adding the term  $0.56H^{2.55}$  to the formula for the discharge through the 0.5-foot rectangular notch, the formula for the discharge through a 0.5-foot Cipolletti notch becomes

$$Q = 1.566H^{1.504} + 0.56H^{2.55}$$

#### NOTCHES WITH SIDE SLOPES OF 1 TO 3 AND 1 TO 6

Experiments were made with notches having crest lengths of 2 feet and side slopes of 1 to 3 and 1 to 6, respectively. Since notches of only one length were used in each set of experiments, no general equations were deduced for notches of these types. The discharges obtained in the experiments for heads over 0.4 foot are shown graphically in figure 11. Discharges with heads less than 0.4 foot are approximately the same as those given in Tables II and VI.

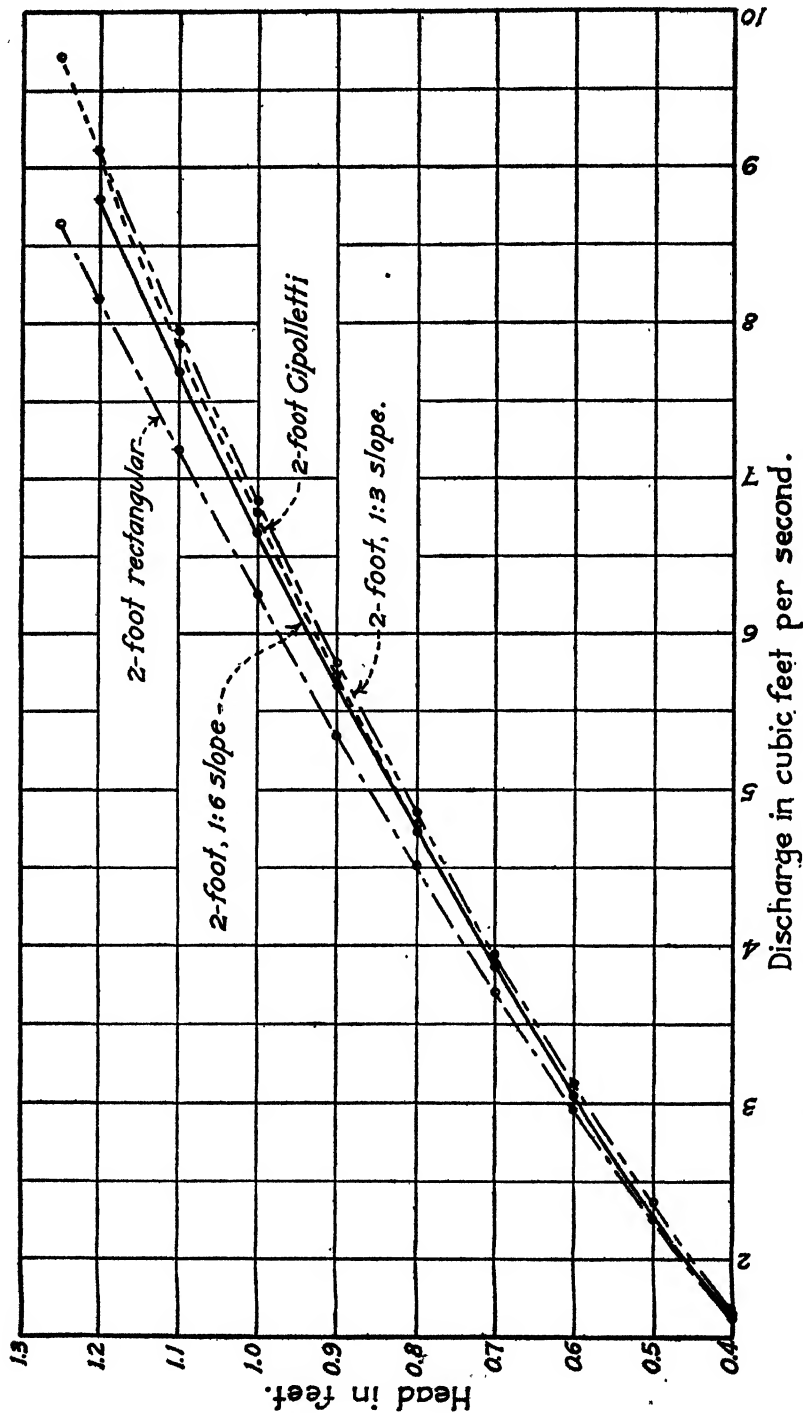


FIG. 11.—Curves showing discharges through 2-foot rectangular and Cipolletti notches and 2-foot notches having 1 to 3 and 1 to 6 side slopes.

## TRIANGULAR NOTCHES

General theoretical formulas have been given for triangular notches (7, p. 46; 8, p. 168), and experiments with a  $90^\circ$  notch have been made by Thomson<sup>1</sup> (12, p. 181; 13, p. 154) and Barr.<sup>2</sup> In the Fort Collins laboratory 98 tests were made with heads ranging from 0.2 foot to 1.35 feet on weirs having triangular notches of  $120^\circ$ ,  $90^\circ$ ,  $60^\circ$ ,  $30^\circ$  and approximately  $28^\circ 4'$ . The side slopes for the last-named notch are 1 horizontal to 4 vertical, and the tests were made with the idea that they might be of use in deriving a formula for discharges through Cipolletti notches.

## DERIVATION OF FORMULAS

The discharges through the different notches when plotted logarithmically gave straight lines, as shown in figure 12. The equations for these lines were found to be as shown in Table IX.

TABLE IX.—Equations for straight lines representing discharges through triangular notches

Notch angle.	Slope of sides, horizontal vertical.	Equation of line.
$120^\circ$	1. 732	$Q=4.400H^{2.4870}$
$90^\circ$	1. 000	$Q=2.487H^{2.4805}$
$60^\circ$	. 577	$Q=1.446H^{2.4705}$
$30^\circ$	. 268	$Q=0.6848H^{2.4476}$
$28^\circ 4' a$	. 250	$Q=0.6405H^{2.4448}$

<sup>a</sup>Approximate.

The discharging streams had a free fall in all the tests except those for the  $120^\circ$  notch. The upper portion of the stream over the  $120^\circ$  notch adhered to the edge of the notch for a distance of approximately 0.1 foot, the distance being quite uniform for all heads. The sides and crest of the notch used were of brass one-fourth inch thick, and were dressed at an angle of about  $45^\circ$  to a thickness of about one thirty-second inch at the edge. As the amount of adherence of nappe for the  $120^\circ$  notch depends upon the thickness of the edges of the notch, the use of such a notch is impracticable.

The data for the  $120^\circ$  notch having been excluded, the general formula for the discharge through the triangular notches of  $28^\circ 4'$  to  $90^\circ$  was found to be

$$Q = (0.025 + 2.462 S) H^{(2.5 - \frac{0.0195}{50.76})}$$

<sup>1</sup> The formula derived by Thomson for the  $90^\circ$  notch was  $Q=0.305H^{2/3}$ , in which  $Q$  is in cubic feet per minute and  $H$  is in inches.

<sup>2</sup> Barr found that with heads of 2 to 10 inches the coefficient  $C$  in Thomson's formula ( $Q=CH^{5/3}$ ) varied from .3104 to .2995. Strickland found that Barr's coefficient  $C$  for any head could be computed from the formula  $C=0.2907+\frac{0.028}{\sqrt{h}}$ ,  $h$  being in inches.

in which  $Q$  is the discharge in cubic feet per second,  $S$  is the slope of the sides, expressed decimally, and  $H$  is the head in feet.

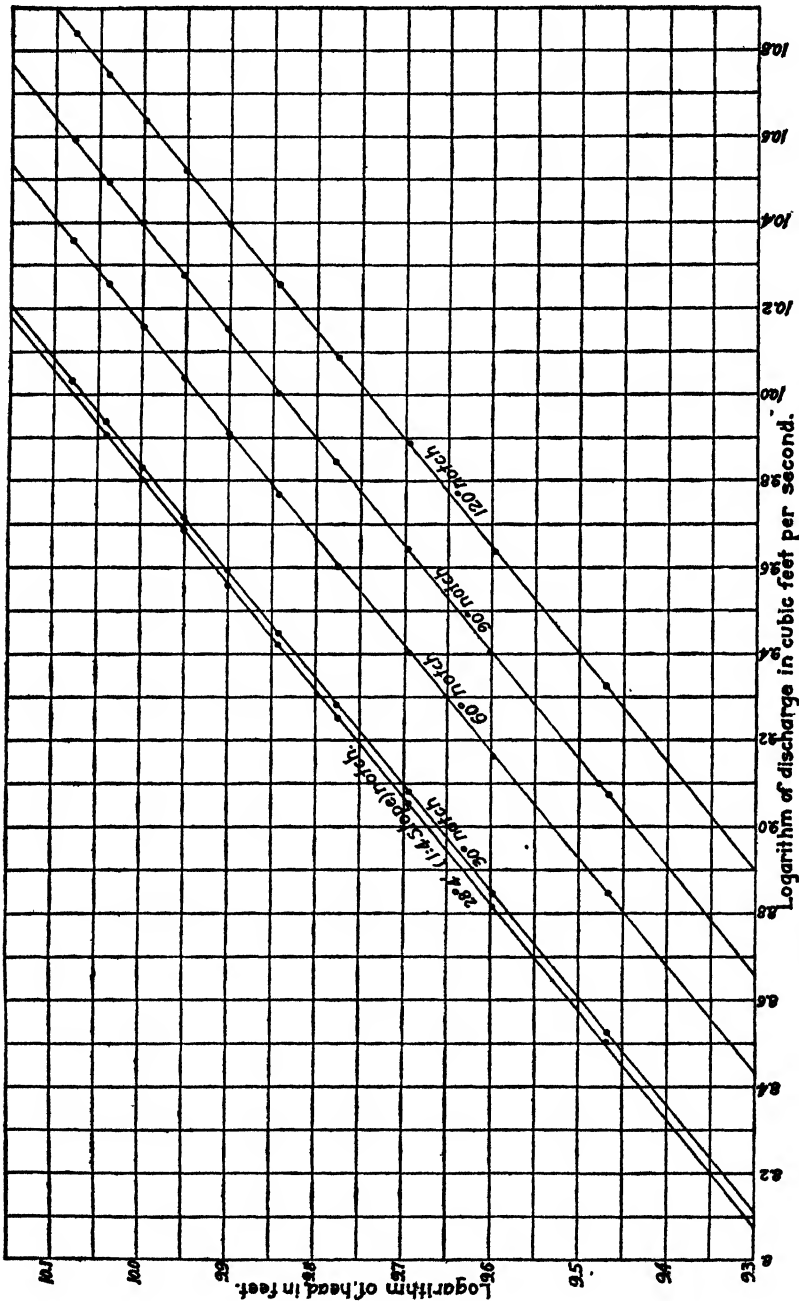


FIG. 12.—Logarithmic diagram of discharges through 28°, 30°, 60°, 90°, and 120° triangular notches.

No experiments were made with notches between 90° and 120°, but a study of the working of the 120° notch led to the conclusion that the

application of the general formula given above can be extended to notches having side slopes of 1 to 1.4 ( $109^\circ$  approximately).

Table X, computed by the new general formula, gives the discharges through notches of different shapes with heads up to 1.25 feet.

TABLE X.—Discharges (in cubic feet per second) for triangular weir notches<sup>1</sup>

Head.		Notch angle $28^\circ 4'$	Notch angle $30^\circ$	Notch angle $60^\circ$	Notch angle $90^\circ$
Feet.	Inches.				
0.20	$2\frac{3}{8}$	0.012	0.013	0.027	0.046
.21	$2\frac{1}{2}$	.014	.015	.031	.052
.22	$2\frac{5}{8}$	.016	.017	.034	.058
.23	$2\frac{3}{4}$	.018	.019	.038	.065
.24	$2\frac{7}{8}$	.020	.021	.043	.072
.25	3	.022	.023	.047	.080
.26	$3\frac{1}{8}$	.024	.025	.052	.088
.27	$3\frac{1}{4}$	.026	.028	.057	.096
.28	$3\frac{3}{8}$	.029	.030	.062	.105
.29	$3\frac{1}{2}$	.031	.033	.068	.115
.30	$3\frac{5}{8}$	.034	.036	.074	.125
.31	$3\frac{3}{4}$	.037	.039	.080	.136
.32	$3\frac{7}{8}$	.040	.042	.087	.147
.33	$3\frac{1}{2}$	.043	.045	.094	.159
.34	$4\frac{1}{8}$	.046	.049	.101	.171
.35	$4\frac{1}{4}$	.049	.052	.108	.184
.36	$4\frac{3}{8}$	.053	.056	.116	.197
.37	$4\frac{1}{2}$	.056	.060	.124	.211
.38	$4\frac{5}{8}$	.060	.064	.132	.225
.39	$4\frac{3}{4}$	.064	.068	.141	.240
.40	$4\frac{7}{8}$	.068	.073	.150	.256
.41	$4\frac{1}{2}$	.072	.077	.160	.272
.42	$5\frac{1}{8}$	.077	.082	.170	.289
.43	$5\frac{1}{4}$	.081	.087	.180	.306
.44	$5\frac{3}{8}$	.086	.092	.190	.324
.45	$5\frac{1}{2}$	.091	.097	.201	.343
.46	$5\frac{5}{8}$	.096	.102	.212	.362
.47	$5\frac{3}{4}$	.101	.108	.224	.382
.48	$5\frac{7}{8}$	.106	.114	.236	.403
.49	$5\frac{1}{2}$	.112	.120	.248	.424
.50	6	.118	.126	.261	.445
.51	$6\frac{1}{8}$	.123	.132	.274	.468
.52	$6\frac{1}{4}$	.129	.138	.287	.491
.53	$6\frac{3}{8}$	.136	.145	.301	.515
.54	$6\frac{1}{2}$	.142	.152	.315	.539
.55	$6\frac{5}{8}$	.148	.159	.330	.564
.56	$6\frac{3}{4}$	.155	.166	.345	.590
.57	$6\frac{7}{8}$	.162	.173	.360	.617
.58	$6\frac{1}{2}$	.169	.181	.376	.644
.59	$7\frac{1}{8}$	.176	.188	.392	.672

<sup>1</sup> Computed by the formula  $Q = (0.025 + 2.462S)H^{2.5 - \frac{0.0195}{S^{0.75}}}$

TABLE X.—Discharges (in cubic feet per second) for triangular weir notches—Continued

Head.		Notch angle 28° 4'	Notch angle 30°	Notch angle 60°	Notch angle 90°
Feet.	Inches.				
0.60	7 $\frac{1}{8}$	0.184	0.196	0.409	0.700
.61	7 $\frac{1}{8}$	.191	.204	.426	.730
.62	7 $\frac{1}{8}$	.199	.212	.444	.760
.63	7 $\frac{1}{8}$	.207	.221	.462	.790
.64	7 $\frac{1}{8}$	.215	.230	.480	.822
.65	7 $\frac{1}{8}$	.223	.239	.499	.854
.66	7 $\frac{1}{8}$	.232	.248	.518	.887
.67	8 $\frac{1}{8}$	.241	.257	.537	.921
.68	8 $\frac{1}{8}$	.250	.266	.557	.955
.69	8 $\frac{1}{4}$	.259	.276	.578	.991
.70	8 $\frac{3}{8}$	.268	.286	.599	1.03
.71	8 $\frac{1}{2}$	.277	.296	.620	1.06
.72	8 $\frac{5}{8}$	.287	.306	.642	1.10
.73	8 $\frac{3}{4}$	.297	.317	.664	1.14
.74	8 $\frac{7}{8}$	.307	.328	.687	1.18
.75	9	.317	.339	.710	1.22
.76	9 $\frac{1}{8}$	.327	.350	.734	1.26
.77	9 $\frac{1}{4}$	.338	.361	.758	1.30
.78	9 $\frac{3}{8}$	.349	.373	.782	1.34
.79	9 $\frac{1}{2}$	.360	.385	.807	1.39
.80	9 $\frac{5}{8}$	.371	.397	.833	1.43
.81	9 $\frac{3}{4}$	.383	.409	.859	1.48
.82	9 $\frac{7}{8}$	.394	.421	.885	1.52
.83	10 $\frac{1}{8}$	.406	.434	.912	1.57
.84	10 $\frac{1}{4}$	.418	.447	.940	1.61
.85	10 $\frac{3}{8}$	.430	.460	.968	1.66
.86	10 $\frac{1}{2}$	.443	.473	.996	1.71
.87	10 $\frac{5}{8}$	.456	.487	1.02	1.76
.88	10 $\frac{3}{4}$	.469	.501	1.05	1.81
.89	10 $\frac{7}{8}$	.482	.515	1.08	1.86
.90	10 $\frac{1}{2}$	.495	.529	1.11	1.92
.91	10 $\frac{1}{2}$	.509	.544	1.15	1.97
.92	11 $\frac{1}{8}$	.522	.558	1.18	2.02
.93	11 $\frac{1}{4}$	.536	.573	1.21	2.08
.94	11 $\frac{1}{4}$	.551	.589	1.24	2.13
.95	11 $\frac{3}{8}$	.565	.604	1.27	2.19
.96	11 $\frac{1}{2}$	.580	.620	1.31	2.25
.97	11 $\frac{5}{8}$	.595	.636	1.34	2.31
.98	11 $\frac{3}{4}$	.610	.652	1.38	2.37
.99	11 $\frac{7}{8}$	.625	.668	1.41	2.43
1.00	12	.641	.685	1.45	2.49
1.01	12 $\frac{1}{8}$	.656	.702	1.48	2.55
1.02	12 $\frac{1}{4}$	.672	.719	1.52	2.61
1.03	12 $\frac{3}{8}$	.688	.736	1.56	2.68
1.04	12 $\frac{1}{2}$	.705	.754	1.59	2.74
1.05	12 $\frac{5}{8}$	.722	.772	1.63	2.81
1.06	12 $\frac{3}{4}$	.739	.790	1.67	2.87
1.07	12 $\frac{7}{8}$	.756	.808	1.71	2.94
1.08	13 $\frac{1}{8}$	.773	.827	1.75	3.01
1.09	13 $\frac{1}{4}$	.791	.846	1.79	3.08

TABLE X.—Discharges (in cubic feet per second) for triangular weir notches—Con.

Head.		Notch angle 28° 4'	Notch angle 30°.	Notch angle 60°.	Notch angle 90°.
Feet.	Inches.				
1. 10	13 $\frac{3}{8}$	0. 809	0. 865	1. 83	3. 15
1. 11	13 $\frac{1}{2}$	. 827	. 884	1. 87	3. 22
1. 12	13 $\frac{1}{4}$	. 845	. 904	1. 91	3. 30
1. 13	13 $\frac{1}{8}$	. 864	. 924	1. 96	3. 37
1. 14	13 $\frac{1}{8}$	. 882	. 944	2. 00	3. 44
1. 15	13 $\frac{1}{8}$	. 901	. 964	2. 04	3. 52
1. 16	13 $\frac{1}{8}$	. 921	. 985	2. 09	3. 59
1. 17	14 $\frac{1}{8}$	. 940	1. 01	2. 13	3. 67
1. 18	14 $\frac{1}{8}$	. 960	1. 03	2. 18	3. 75
1. 19	14 $\frac{1}{4}$	. 980	1. 05	2. 22	3. 83
1. 20	14 $\frac{3}{8}$	1. 00	1. 07	2. 27	3. 91
1. 21	14 $\frac{1}{2}$	1. 02	1. 09	2. 32	3. 99
1. 22	14 $\frac{3}{8}$	1. 04	1. 11	2. 36	4. 07
1. 23	14 $\frac{3}{4}$	1. 06	1. 14	2. 41	4. 16
1. 24	14 $\frac{1}{8}$	1. 08	1. 16	2. 46	4. 24
1. 25	15	1. 11	1. 19	2. 51	4. 33

Although weirs with triangular notches are well suited to a comparatively wide range of discharges, they are especially well adapted for the measurement of small discharges and may be used to measure accurately quantities so small that they would not pass through trapezoidal or rectangular notches without adhering to the crests. The use of weirs with triangular notches requires slightly more fall than is required with trapezoidal or rectangular notches—that is, a head of 2 feet is required to deliver approximately 14 cubic feet per second through a 90° triangular notch, while the same discharge would be delivered through a 3-foot rectangular notch with a head of 1.31 feet, or through a 4-foot rectangular notch with a head of 1.07 feet.

Weirs with 90° notches are simpler in construction than any other type of weir and are the most practical type for small or medium-sized discharges. The approximate formula  $Q = 2.49H^{2.48}$  gives discharge values for 90° notches, which agree very closely with the values obtained with the general formula.



## COMPARISON OF NEW FORMULA AND OLD FORMULA

The discharges for the 90° notch computed by the new and the old formulas are compared in Table XI:

TABLE XI.—Comparison of new formula and old formula

Head.	Discharge computed by new formula (cubic feet per second).	Discharge computed by old formula, $Q = 2.53H^{3/2}$ .	
		Discharge in cubic feet per second.	Percentage of discharge computed by new formula.
<i>Feet</i>			
0.20	0.046	0.045	97.8
.33	.159	.158	99.4
.50	.445	.447	100.4
.67	.921	.930	101.0
.85	1.66	1.69	101.8
1.00	2.49	2.53	101.6
1.25	4.33	4.42	102.1

As no experiments have been made in the past to determine the coefficients in general formulas for notches of 28° 4', 30°, or 60°, no comparison could be made with the discharges through such notches computed with the new formula.

## CIRCULAR NOTCHES

Apparently no experiments have ever been made with circular or semi-circular notches placed in a vertical position with heads less than the height of the opening. In order to throw light upon the probable discharges through such notches and obtain data to use in determining the flow through circular head gates when acting as weirs rather than as orifices, 50 tests were made with thin-edged circular notches, 17 being with a notch 0.4995 foot in diameter and 33 with a notch 1.0025 feet in diameter; and 34 tests were made with semicircular notches, 15 being with a notch 1.5011 feet in diameter and 19 with a notch 1.9990 feet in diameter. The discharge data obtained are shown graphically in figure 13.

## CONDITIONS OF NOTCH EDGES REQUIRED TO INSURE FREE FLOW

The impression is common that the terms "thin edges" and "sharp crests," as applied to weir notches, mean knife edges. Such edges are not necessary, and the edges are sufficiently sharp or thin if the upstream corner of the notch edges is a distinct angle of 90° or less and the thickness of the notch edges is not so great that the water will adhere to them. The allowable thickness of the edges depends upon the head that is being used. Experiments made in the laboratory with notches having edges

$\frac{1}{4}$  inch thick showed that while water would adhere to the notch edges

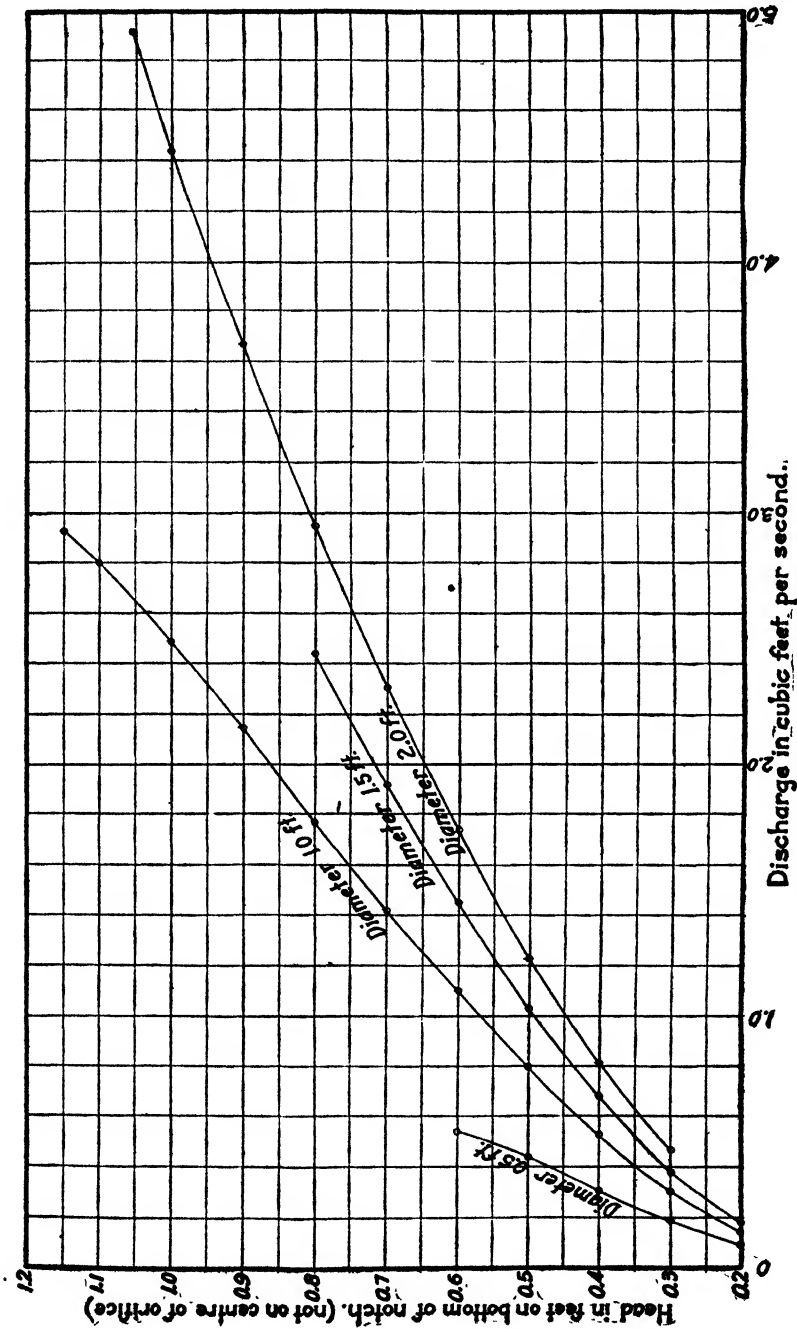


FIG. 13.—Curves showing discharges through circular weir notches.

with a head of 0.15 foot, there was no adherence with heads of 0.2 foot and over.

Notches with angles made as precisely as those used in the test would not be practicable for field use, and consequently a maximum thickness of  $\frac{1}{8}$  inch probably would be safer than  $\frac{1}{4}$  inch where heads as low as 0.2 foot will be used. While no experiments were made, edges as thick as  $\frac{3}{4}$  inch probably can be used where the minimum head will be 1 foot.

The edges of the weir notches must be straight, true, and rigid. These conditions are best insured by using angle irons or similar material that can be securely fastened to the bulkheads, as wood edges become splintered and warped, and thin sheet-metal weir plates buckle and bend easily. Regardless of the material used, the notches will be more permanent and reliable if the upstream corners of the notches are made definitely angular and the edges are left as thick as possible and still permit a free flow.

#### DISTANCE FROM NOTCH AT WHICH HEAD SHOULD BE MEASURED

In connection with the experiments with notches of different types, measurements were made to determine the transverse and longitudinal curves of the water surface upstream from the weirs when different heads were being used. These measurements showed that the extent of the curves backward from and to the sides of the notches depends upon the length of the crest and the head being used. Plots of the data obtained show that measurements of head should be made either at a distance of at least  $4H$  upstream from the notch or at a distance of at least  $2H$  side-wise from the end of the crest of the notch.

Table XII gives the errors and the percentage of error made in computing discharges for notches of different shapes and sizes with different heads caused by errors of 0.01 foot in reading the heads.

TABLE XII.—Errors and percentage of error in computed discharges caused by 0.01-foot error in reading the heads

#### RECTANGULAR WEIRS

Correct head.	Error.									
	1-foot crest.		1½-foot crest.		2-foot crest.		3-foot crest.		4-foot crest.	
	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.
Feet.										
0.20	0.021	7.22	0.033	7.52	0.044	7.48	0.067	7.55	0.09	7.56
.30	.026	4.94	.04	5.03	.05	4.67	.08	4.97	.10	4.63
.40	.029	3.61	.05	4.13	.06	3.68	.09	3.66	.12	3.64
.50	.04	3.60	.05	2.98	.07	3.10	.10	2.92	.14	3.06
.60	.04	2.76	.05	2.27	.07	2.36	.11	2.46	.14	2.33
.70	.04	2.20	.06	2.17	.07	1.89	.12	2.14	.16	2.13
.80	.04	1.81	.06	1.79	.08	1.77	.12	1.76	.17	1.86
.90	.05	1.91	.07	1.76	.09	1.68	.13	1.60	.18	1.65
1.00	.05	1.63	.07	1.51	.09	1.44	.14	1.48	.19	1.49
1.10	.....	.....	.07	1.31	.09	1.25	.14	1.28	.19	1.30
1.20	.....	.....	.07	1.16	.10	1.21	.15	1.21	.20	1.20
1.30	.....	.....	.....	.....	.....	.....	.16	1.15	.21	1.18
1.40	.....	.....	.....	.....	.....	.....	.16	1.03	.22	1.05
1.50	.....	.....	.....	.....	.....	.....	.16	.93	.23	1.00

TABLE XII.—Errors and percentage of error in computed discharges caused by 0.01-foot error in reading the heads—Continued

## CIPOLLETTI WEIRS

Correct head.	Error.									
	1-foot crest.		1¼-foot crest.		2-foot crest.		3-foot crest.		4-foot crest.	
Feet.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.	Cu. ft. per sec.	Per ct.
0.20	0.022	7.3	0.034	7.6	0.045	7.5	0.068	7.6	0.09	7.5
0.30	0.028	5.0	0.041	5.0	0.055	5.0	0.082	5.0	0.11	5.0
0.40	0.034	3.9	0.05	3.9	0.07	4.1	0.09	3.6	0.12	3.6
0.50	0.04	3.3	0.05	2.8	0.07	3.0	0.11	3.1	0.14	3.0
0.60	0.04	2.5	0.06	2.5	0.08	2.6	0.12	2.6	0.15	2.4
0.70	0.05	2.4	0.07	2.3	0.09	2.3	0.13	2.2	0.17	2.2
0.80	0.05	2.0	0.07	1.9	0.09	1.9	0.14	1.9	0.18	1.9
0.90	0.05	1.6	0.08	1.8	0.10	1.7	0.15	1.7	0.19	1.7
1.00	0.06	1.6	0.08	1.5	0.11	1.6	0.15	1.5	0.20	1.5
1.10	.....	.....	0.09	1.5	0.12	1.5	0.17	1.5	0.21	1.4
1.20	.....	.....	0.09	1.3	0.12	1.3	0.17	1.3	0.22	1.3
1.30	.....	.....	.....	.....	.....	.....	0.18	1.2	0.24	1.2
1.40	.....	.....	.....	.....	.....	.....	0.19	1.1	0.24	1.1

## 90° TRIANGULAR WEIRS

0.20	0.006	13.04	.....	.....	.....	.....	.....	.....	.....
0.50	0.022	4.94	.....	.....	.....	.....	.....	.....	.....
0.70	0.04	3.9	.....	.....	.....	.....	.....	.....	.....
1.00	0.06	2.4	.....	.....	.....	.....	.....	.....	.....
1.25	0.09	2.1	.....	.....	.....	.....	.....	.....	.....

## EFFECTS OF DIFFERENT END AND BOTTOM CONTRACTIONS UPON DISCHARGES

## RECTANGULAR AND CIPOLLETTI NOTCHES

To determine the effect of different end and bottom contractions upon the discharges through rectangular and Cipolletti notches, 120 tests were made with 1-foot rectangular notches, 72 with 3-foot rectangular notches, 205 with 1-foot Cipolletti notches, and 89 with 3-foot Cipolletti notches. Heads of 0.2 foot, 0.6 foot, and 1 foot were used with each notch. The end contractions (the distances of the sides of the weir box from the ends of the crest) and the bottom contraction (the distance of the bottom of the weir box below the crest of the notch) for each notch were varied from 0.5 foot to 3 feet by increments of 0.5 foot. The discharges under the different conditions were compared with those obtained with the standard weir box. The small error in the experimental determinations of the discharges with a 0.2-foot head caused such large percentages of error in the discharges that they were unreliable and so were not included.

Figures 14 and 15 and Tables XIII and XIV show the percentages of increase in discharges and the velocities of approach with heads of 0.6 foot and 1 foot under the different conditions of contractions. The equations of the curve are all of the general form,  $e = a(V + b)^n$ , in which  $e$

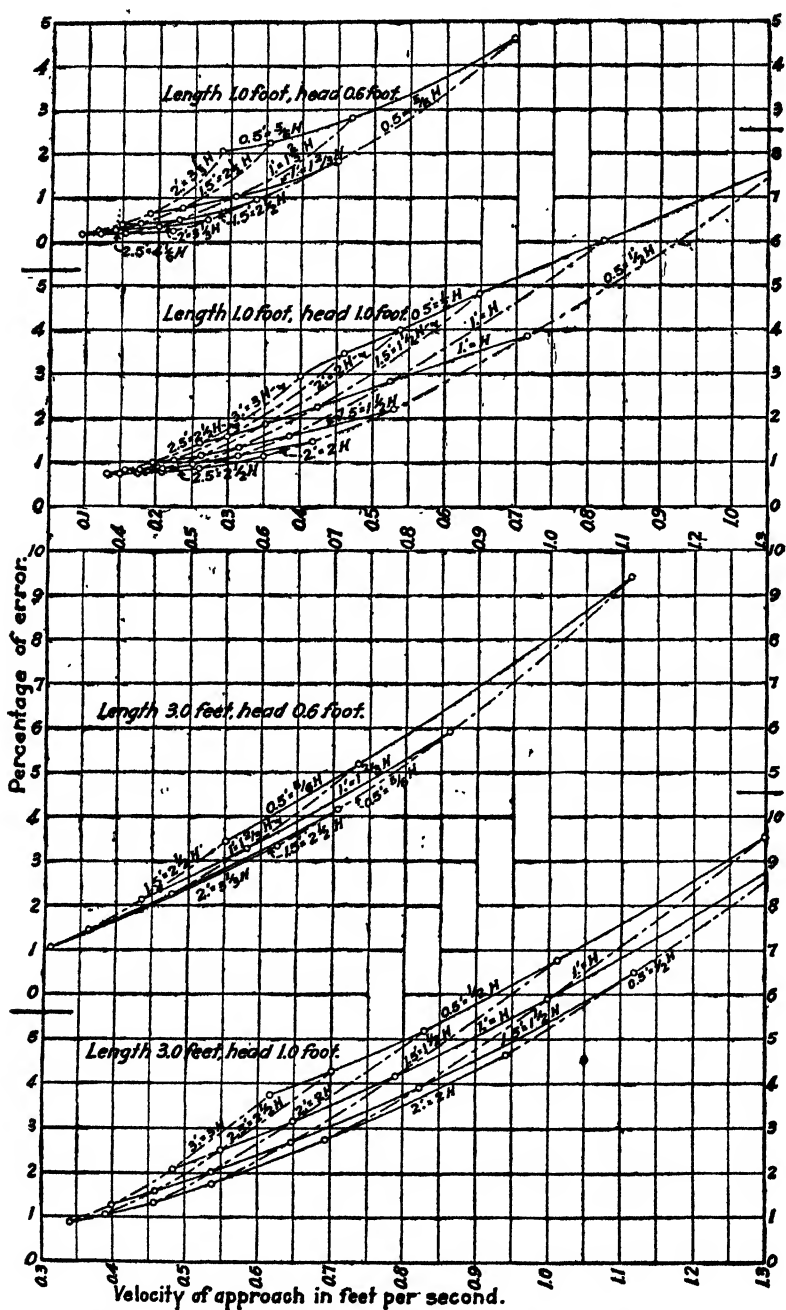


FIG. 14.—Curves showing effect of different end and bottom contractions upon discharges through 1-foot and 3-foot rectangular notches with heads of 0.6 and 1 foot. Full lines show end contractions; dot-dash lines show side contractions.

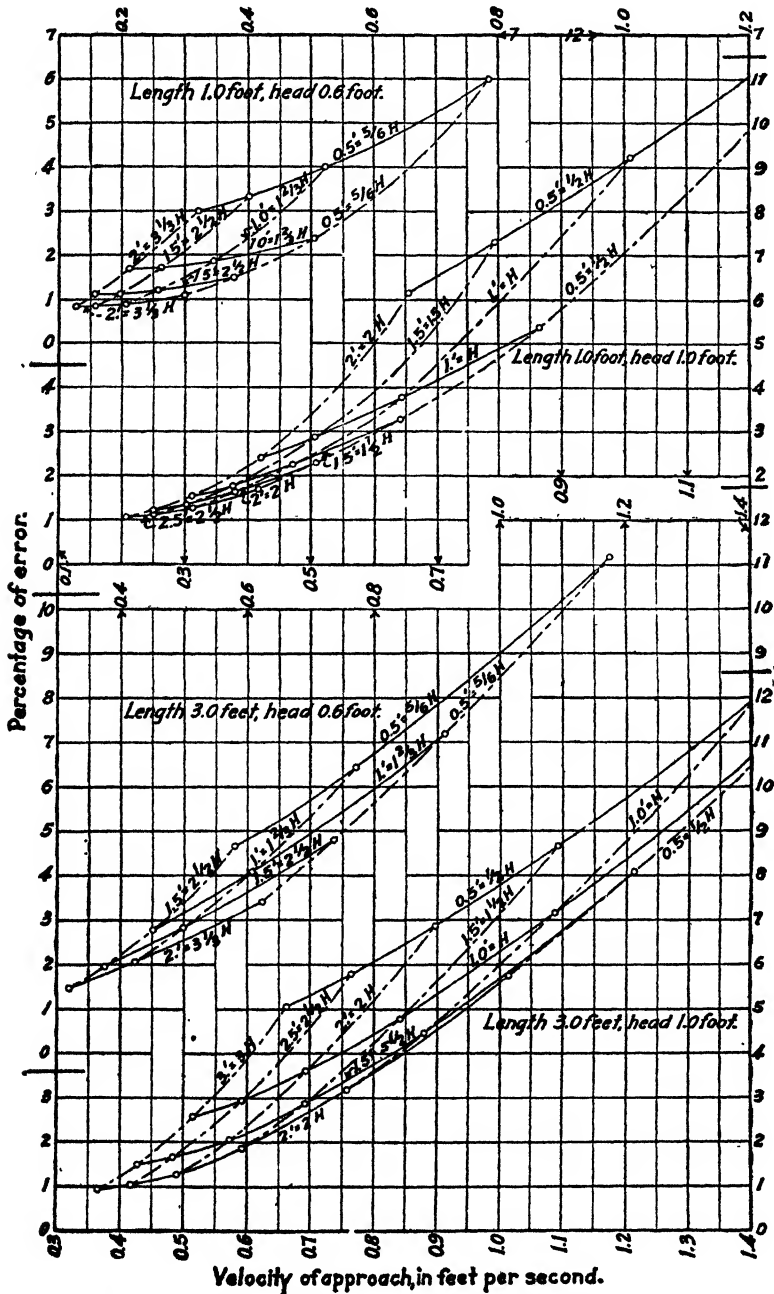


FIG. 15.—Curves showing the effect of different end and bottom contractions upon the discharges through 1-foot and 3-foot Cipolletti weir notches with heads of 0.6 and 1 foot. Full lines show end contractions in feet; dot-dash lines show bottom contractions in feet.

is the percentage of increase in discharge,  $V$  is the average velocity of approach, and  $a$ ,  $b$ , and  $n$  are constants for each size of each type of notch.

TABLE XIII.—*Velocities of approach (in feet per second) and percentages of increase in discharges through rectangular notches caused by different end and bottom contractions*

HEAD, 0.6 FOOT

Bottom contraction.	End contractions.	1-foot notch.		1½-foot notch.		2-foot notch.		3-foot notch.		4-foot notch.	
		Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.
Feet.	Feet.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
2	2.5	0.094	0.17								
	2.0	.115	.26								
	1.5	.148	.39								
	1.0	.188	.66								
	.5	.288	2.05								
1½	2.5	.119	.17								
	2.0	.141	.30	0.191	0.53	0.239	0.74	0.308	1.07	0.365	1.33
	1.5	.175	.40	.234	.73	.286	1.01	.363	1.44	.416	1.74
	1.0	.234	.76	.304	1.24	.361	1.62	.435	2.12	.489	2.49
	.5	.355	2.26					.552	3.41		
1	2.5	.154	.19								
	2.0	.209	.36								
	1.5	.229	.50	.311	1.09	.377	1.55	.478	2.25	.552	2.79
	1.0	.308	1.01	.400	1.77	.476	2.39	.577	3.22	.650	3.83
	.5	.469	2.84	.573	3.74	.646	4.38	.735	5.15	.794	5.64
¾	2.5	.221	.25								
	2.0	.268	.50	.368	1.30	.460	2.05	.624	3.34	.711	4.05
	1.5	.337	.94	.453	1.94	.555	2.84	.705	4.17	.818	5.15
	1.0	.450	1.84	.588	3.22	.704	4.35	.862	5.92	.975	7.01
	.5	.695	4.63	.852	6.43	.970	7.79	1.112	9.40	1.208	10.50

HEAD, 1 FOOT

3	2.5	0.132	0.74								
	2.0	.157	.81	0.213	0.82	0.269	0.84	0.342	0.83	0.402	0.87
	1.5	.196	.99	.260	1.08	.317	1.14	.399	1.22	.460	1.29
	1.0	.260	1.40	.337	1.63	.398	1.81	.484	2.06	.543	2.22
	.5	.40	2.94	.477	3.22	.540	3.44	.616	3.72	.661	3.88
2½	2.5	.150	.74								
	2.0	.178	.82	.242	.88	.302	.94	.391	1.04	.460	1.11
	1.5	.224	1.05	.297	1.21	.362	1.34	.461	1.57	.528	1.69
	1.0	.299	1.58	.385	1.89	.457	2.14	.553	2.50	.623	2.76
	.5	.462	3.42	.549	3.73	.625	3.99	.704	4.25	.760	4.48
2	2.5	.175	.73								
	2.0	.209	.84	.284	.97	.352	1.11	.458	1.30	.539	1.42
	1.5	.261	1.13	.348	1.42	.424	1.67	.538	2.01	.620	2.28
	1.0	.353	1.83	.450	2.28	.535	2.63	.648	3.14	.733	3.52
	.5	.538	4.01	.646	4.46	.728	4.80	.829	5.17	.895	5.47
1¾	2.5	.208	.74								
	2.0	.252	.94	.341	1.18	.424	1.41	.539	1.71	.638	1.98
	1.5	.314	1.31	.418	1.74	.512	2.12	.648	2.65	.750	3.07
	1.0	.424	2.24	.544	2.87	.646	3.40	.790	4.14	.889	4.68
	.5	.648	4.80	.784	5.53	.885	6.09	1.013	6.77	1.091	7.20
1½	2.5	.260	.82								
	2.0	.314	1.12	.427	1.57	.532	2.00	.694	2.69	.810	3.15
	1.5	.385	1.59	.528	2.37	.645	2.99	.820	3.91	.952	4.60
	1.0	.525	2.53	.688	3.86	.825	4.73	.999	5.87	1.135	6.77
	.5	.822	6.00	.994	7.29	1.129	8.29	1.298	9.55	1.405	10.27
¾	2.5	.350	1.11								
	2.0	.417	1.45	.575	2.40	.720	3.27	.845	4.62	1.120	5.65
	1.5	.530	2.20	.710	3.53	.875	4.73	1.110	6.50	1.308	7.88
	1.0	.716	3.83	.930	5.65	1.118	7.28	1.380	9.40	1.576	11.2
	.5	1.120	8.25	1.37	11.0	1.58	13.5	1.83	16.01	2.01	18.0

TABLE XIV.—*Velocities of approach (in feet per second) and percentages of increase in discharges through Cipolletti notches caused by different bottom and end contractions*

## HEAD, 0.6 FOOT

Bottom contraction.	End contractions.	1-foot notch.		1½-foot notch.		2-foot notch.		3-foot notch.		4-foot notch.	
		Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.	Velocity of approach.	Increase of discharge.
Feet.	Feet.		Per ct.		Per ct.		Per ct.		Per ct.		Per ct.
1½ .....	2.0	0.158	0.84	0.207	1.02	0.251	1.21	0.321	1.45	0.373	1.61
	1.5	.196	1.11	.255	1.38	.304	1.60	.377	1.95	.429	2.19
	1.0	.260	1.70	.329	2.08	.381	2.36	.454	2.77	.504	3.02
	.5	.400	3.32	.469	3.83	.518	4.20	.580	4.66	.617	4.93
1 .....	2.0	.205	.90	.274	1.25	.331	1.55	.425	2.05	.492	2.39
	1.5	.257	1.20	.335	1.71	.400	2.17	.500	2.82	.569	3.30
	1.0	.344	1.84	.434	2.60	.501	3.17	.607	4.06	.671	4.60
	.5	.529	4.00	.622	4.92	.690	5.61	.770	6.41	.826	6.98
½ .....	2.0	.300	1.11	.399	1.81	.487	2.42	.625	3.40	.725	4.09
	1.5	.377	1.51	.492	2.55	.589	3.44	.737	4.79	.847	5.80
	1.0	.505	2.39	.636	3.93	.750	5.30	.908	7.18	1.013	8.39
	.5	.782	6.03	.932	8.02	1.037	9.43	1.173	11.28	1.263	12.48

## HEAD, 1 FOOT

2 .....	2.0	0.250	1.19	0.322	1.22	0.386	1.24	0.488	1.28	0.561	1.30
	1.5	.314	1.52	.397	1.70	.467	1.84	.575	2.08	.648	2.22
	1.0	.422	2.40	.514	2.80	.590	3.15	.698	3.62	.769	3.92
	.5	.655	6.16	.746	6.41	.813	6.61	.896	6.88	.951	7.01
1½ .....	2.0	.300	1.34	.388	1.49	.465	1.61	.590	1.82	.680	1.98
	1.5	.378	1.78	.477	2.10	.562	2.40	.693	2.85	.785	3.17
	1.0	.508	2.89	.622	3.53	.714	4.06	.844	4.79	.937	5.31
	.5	.795	7.29	.906	7.79	.989	8.18	1.094	8.64	1.163	8.95
1 .....	2.0	.374	1.60	.489	2.06	.586	2.44	.758	3.13	.864	3.55
	1.5	.471	2.20	.601	2.92	.710	3.55	.888	4.52	1.003	5.19
	1.0	.643	3.76	.787	4.83	.908	5.73	1.083	7.07	1.200	7.92
	.5	1.070	9.20	1.159	10.28	1.271	11.08	1.410	12.09	1.503	12.72
½ .....	2.5	.64	3.3	.818	4.8	.968	6.07	1.21	8.07	1.391	9.56
	2.0	.508	2.30	.660	3.64	.799	4.87	1.013	6.73	1.202	8.39
	1.5	.640	3.30	.818	4.80	.969	6.09	1.210	8.08	1.391	9.56
	1.0	.864	5.40	1.077	7.58	1.258	9.43	1.505	11.95	1.688	13.82
	.5	1.390	11.89	1.605	14.63	1.782	16.85	2.015	19.80	....	....

Figure 16 shows the variation of the percentages of increase in the discharges through a 1-foot rectangular notch, with heads of 0.6 foot and 1 foot as the ratio of the cross-sectional area of the weir box ( $A$ ) to the area of the weir notch ( $a$ ), decreased with the use of different end and bottom contractions. From these curves it will be seen that changing the position of the sides of the weir box and leaving the bottom in a fixed position has a greater effect upon the discharges than leaving the sides fixed and moving the bottom. This indicates that end contractions have more effect upon the discharges than do bottom contractions. With end contractions equal to  $2H$  and a bottom contraction equal to  $3H$ , or end contractions equal to  $3H$  and a bottom contraction equal to  $2H$ , the mean velocities of approach are about one-third foot per second and the discharges with medium to high heads do not agree closer than approximately 1 per cent with the discharges computed by the formula.



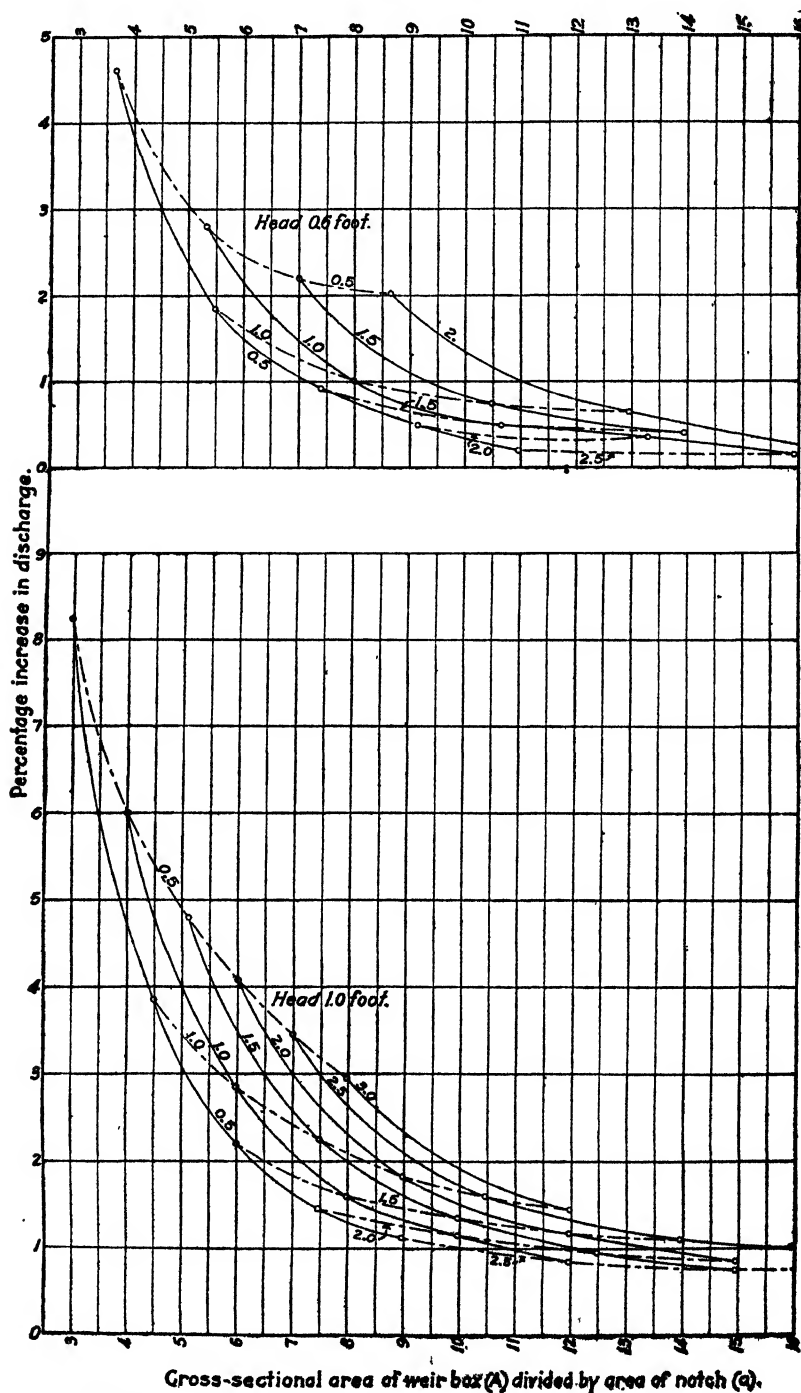


FIG. 16.—Curves showing the effect of different ratios of cross-sectional area of the weir box (A) to the area of the notch (a) upon discharges through a 1-foot rectangular notch with heads of 0.6 foot and 1 foot. Full lines show bottom contractions in feet; dot-dash lines show end contractions in feet.

This indicates that a mean velocity of one-third foot per second is allowable where an error of 1 per cent in discharge is permissible.

By superimposing upon the similar curves for Cipolletti notches the curves showing the effect of different end and bottom contractions upon the discharges through rectangular notches, it was found that the end-contraction distances for Cipolletti notches should be taken from about the middle point of the side of the notch instead of from the end of the crest, in order to make the results of the two types of notches comparable.

Since the minimum bottom and end contractions possible without increasing the discharges beyond an allowable limit increase with the increase of the head run, weir boxes should be designed so as to give discharges within the allowable limit when the highest head intended to be run over the notch is being run. Francis stated (5, p. 72 and 134):

In order that the end contraction may be complete, the sill and sides of the weir must be so far removed from the bottom and lateral sides of the reservoir (weir box) that they may produce no more effect upon the discharge than if they were removed a distance infinitely great.

He concludes from his experiments that an end contraction of  $1H$  and a bottom contraction of  $2H$  are the least permissible in order that his formula may apply.

Smith (10, p. 120) gave the necessary end contractions as  $3H$ . He also suggested (p. 122) that the effect of contraction should not be confused with the effect of velocity of approach, which is so commonly done in taking the term "complete contraction" to include both the effect of contraction and the velocity of approach. Cipolletti (3, p. 23-24) accepted the results of the Francis experiments for end and bottom contractions. He also quotes a rule deduced by Lesbros from results of his (Lesbros's) experiments, that both contractions should be at least 2.7 times the depth of the nappe. Cipolletti (3), from the experiments of Francis (5), deduced the following: (1) When the end contractions equal  $2H$  and the bottom contraction  $3H$ , the bottom and side walls no longer have any appreciable effect upon the discharges through the notch. This condition, he states, may cause an increase of about 0.15 per cent in the discharge. (2) With end contractions of  $1.5H$  and a bottom contraction of  $2.5H$  the increase in discharge would be about 0.5 per cent. (3) With end contractions of  $1H$  and a bottom contraction of  $2H$  the discharges will be increased about 1 per cent. He also takes account of the fact that the velocity of approach must not exceed a certain limit.

The ratio of the cross-sectional area of the weir box to the cross-sectional area of the notch necessary for complete contraction has been given by Carpenter (2, p. 29) as 7. The coefficient using this expression of ratio was proposed by J. Weisbach<sup>6</sup> in 1845 and has been elaborated upon by a number of writers and experimenters (6, p. 312). Figure 16 indicates that there is no fixed value of the ratio  $A$  to  $a$  which will insure

complete contraction in all cases. It also indicates that the value of such ratio should be greater than 7 in all cases, and that 15 probably would come nearer than 7 to meeting average conditions.

#### EFFECT OF SUPPRESSING BOTTOM CONTRACTIONS WITH A 90° TRIANGULAR NOTCH

In order to throw more light upon the question of the effect of bottom contractions upon discharges through triangular notches (9, p. 114-116) experiments were made with a 90° triangular notch with the floor of the weir box at the same level as the vertex of the notch. The width of the weir box used was 10 feet, being the same as that in the standard test with complete contractions, but in the standard test the floor was about 4½ feet below the vertex of the notch. The discharges through the 90° triangular notch with the bottom contraction entirely suppressed was found to be represented by the formula  $Q = 2.53H^{2.490}$ , which varies but little from Thomson's formula for the flow through a 90° triangular notch having complete bottom contractions. It is probable that some part of the increased discharge obtained when the floor was level with the vertex of the notch was due to the increased velocity of approach. The increase in the discharges amounted to 1.6 per cent with a head of 1 foot, but gradually diminished as the head was decreased. The percentage of increase with heads of 0.3 foot or over is represented by the formula  $E = 101.6H^{0.016} - 100$ .

#### RELATION OF LENGTHS OF NOTCHES TO DISCHARGES

The principal advantage claimed in irrigation practice for Cipolletti notches over other notches has been that the discharges are proportional to the crest lengths. This claim is not in accordance with the limitation put on the notch by Francis and Cipolletti, but has been very generally made in irrigation practice. The failure of this theory is shown in Table XV, in which the discharges through Cipolletti and rectangular notches of different lengths are compared with the discharges through a 1-foot Cipolletti and a 1-foot rectangular notch, multiplied by the number of feet in length of the notches. The percentages in the table represent the failure of the larger notches to give discharges proportional to their lengths. It will be seen from the table that rectangular notches give discharges which are more nearly proportional to their lengths than do Cipolletti notches. The percentages of error increase with the head and length of the crest until the discharge through a 4-foot Cipolletti notch with a 1-foot head is 9.2 per cent less than four times the flow through a 1-foot notch with a 1-foot head, and the discharge through a 4-foot rectangular notch is 4 per cent greater than 4 times the discharge through a 1-foot rectangular notch with a 1-foot head. Side slopes of 1 to 4 are therefore too flat and vertical sides are too steep to give discharges proportional to the length of the crest.

TABLE XV.—Relation of length to discharge (in cubic feet per second) of weirs

## RECTANGULAR NOTCHES

Head.  <i>Feet.</i>	1.5-foot crest.				2-foot crest.				3-foot crest.				4-foot crest.			
	Dis- charge.	1.5 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	2 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	3 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	4 X dis- charge through 1-foot notch.	Difference.	
			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.
0.20	0.201	0.439	0.437	0.002	0.588	0.582	0.006	1.0	0.887	0.873	0.014	1.6	1.187	1.164	0.023	2.0
.25	.404	.609	.606	.003	.817	.808	.009	1.1	1.233	1.212	.021	1.7	1.630	1.616	.014	2.1
.30	.527	.796	.790	.006	1.068	1.054	.014	1.3	1.612	1.581	.031	2.0	2.158	2.108	.050	2.4
.40	.824	1.266	1.260	.006	1.630	1.606	.024	1.5	2.404	2.412	.008	2.2	3.299	3.216	.083	2.6
.50	1.113	1.684	1.670	.014	2.262	2.226	.036	1.6	3.421	3.339	.082	2.5	4.583	4.452	.131	2.9
.60	1.453	2.201	2.180	.021	2.936	2.906	.030	1.7	4.474	4.359	.115	2.6	5.906	5.812	.094	3.2
.70	1.819	2.756	2.720	.036	3.618	3.618	.000	1.8	5.611	5.457	.154	2.8	7.522	7.276	.246	3.4
.80	2.210	3.351	3.315	.036	4.320	4.320	.000	1.9	6.828	6.610	.218	3.0	9.158	8.840	.318	3.6
.90	2.624	3.980	3.916	.064	5.354	5.248	.106	2.0	8.118	7.872	.246	3.1	10.891	10.496	.395	3.8
1.00	3.058	4.644	4.587	.057	6.247	6.116	.131	2.1	9.476	9.174	.302	3.3	12.716	12.232	.484	4.0

## CIPOLLETTI NOTCHES

Head.  <i>Feet.</i>	Dis- charge.	1.5 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	2 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	3 X dis- charge through 1-foot notch.	Difference.		Dis- charge.	4 X dis- charge through 1-foot notch.	Difference.	
			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.
			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.			Amount.	Per cent.
0.20	0.302	0.450	0.453	0.003	0.599	0.604	0.005	0.8	0.958	0.906	0.052	5.4	1.198	1.208	0.010	0.8
.25	.423	.628	.634	.006	.816	.816	.000	0.9	1.252	1.269	.017	1.3	1.669	1.692	.023	1.4
.30	.557	.826	.835	.009	1.098	1.114	.016	1.4	1.642	1.671	.029	1.7	2.188	2.228	.040	1.8
.40	.866	1.277	1.299	.022	1.662	1.732	.070	2.3	2.576	2.598	.022	2.8	3.361	3.464	.103	3.0
.50	1.221	1.793	1.831	.038	2.370	2.442	.072	2.9	3.559	3.603	.044	3.7	4.661	4.884	.223	4.0
.60	1.623	2.371	2.434	.063	3.126	3.246	.120	3.7	4.644	4.869	.225	4.6	6.166	6.492	.326	5.0
.70	2.069	3.006	3.103	.097	3.955	4.138	.183	4.4	5.861	6.207	.346	5.6	7.772	8.276	.504	6.1
.80	2.559	3.700	3.838	.138	4.855	5.118	.263	5.1	7.177	7.677	.500	6.5	9.507	10.236	.729	7.1
.90	3.092	4.448	4.638	.190	5.822	6.184	.362	5.9	8.586	9.276	.690	7.4	11.359	12.368	1.009	8.2
1.00	3.667	5.231	5.500	.269	6.836	7.334	.498	6.5	10.085	11.001	.916	8.3	13.325	14.668	1.343	9.2

For the purpose of throwing light upon what would be the probable shape of a type of notch the discharges through which, with the given head, would be proportional to the crest length, the data obtained for the 2-foot rectangular, the 2-foot Cipolletti, and the 2-foot trapezoidal

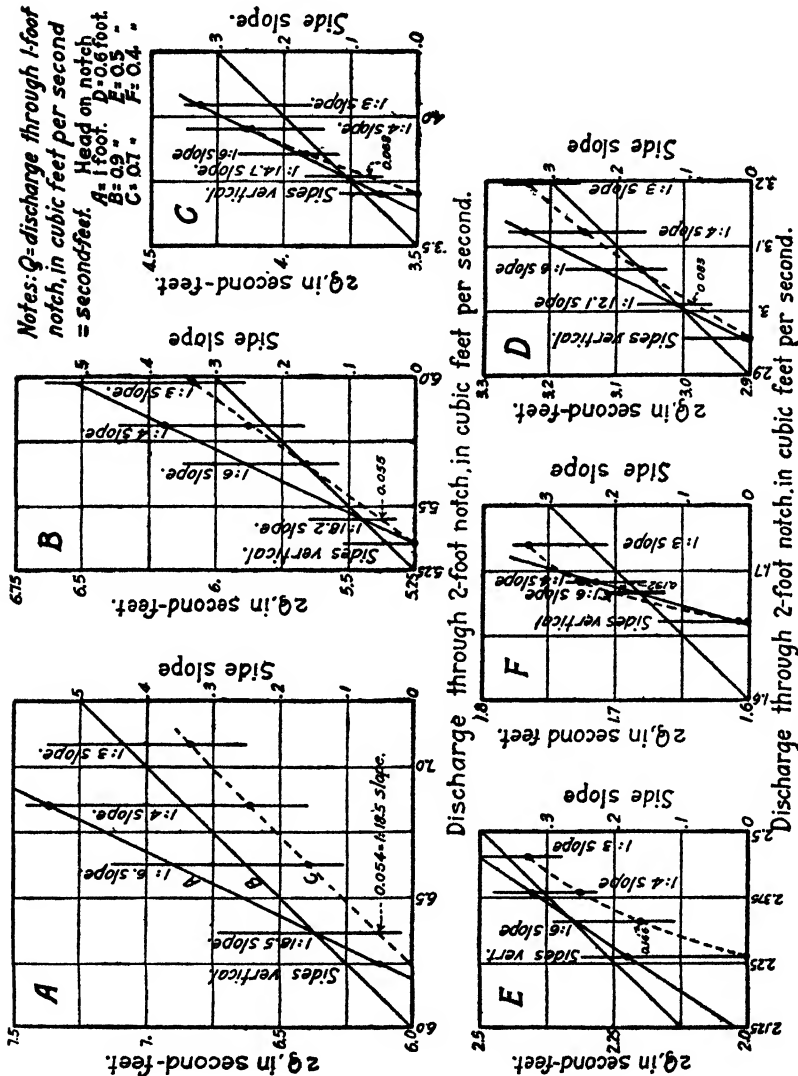


FIG. 17.—Curves showing the side slopes required with different heads in order that the discharge through a 2-foot notch will be twice the discharge through a 1-foot notch.

notches with side slopes 1 to 3 and 1 to 6 were plotted, a set of curves being made for each of the following heads: 0.4, 0.5, 0.6, 0.7, 0.9, and 1 foot (fig. 17). Lines A were obtained by plotting the actual discharges through the rectangular and Cipolletti notches with a given head against twice the discharges through 1-foot notches with the same head. Since no experiments were made with 1-foot notches having side slopes

1 to 3 and 1 to 6, it was assumed that similar plottings for such notches would lie on the same straight line as those for the rectangular and Cipolletti notches. Lines *B* pass through the origin and have a slope of  $45^\circ$ . The discharges through a 2-foot notch with the various heads that would fulfill the condition of being twice the discharge through a 1-foot notch with the same head must lie on this  $45^\circ$  line. Curves *C* were obtained by plotting the discharges through the 2-foot notches of different shapes against the decimal expression of the side slope of the notches.

In each set of curves the point of intersection with the *C* curve of a vertical line drawn through the point of intersection of lines *A* and *B* indicates the side slopes which are necessary with a given head in order that the discharge through a 2-foot notch shall be twice that through a 1-foot notch. The slopes found expressed as ratios of the horizontal to the vertical distance are given in Table XVI and indicate that the sides of a 2-foot notch which would give twice the discharges of a similar 1-foot notch with heads up to 1 foot at least must be curves and must approach the vertical as they go up.

TABLE XVI.—*Side slopes necessary in order that a 2-foot notch discharge twice the amount of water from a 1-foot notch*

Head	Slopes.
<i>Feet.</i>	
1.0	1 to 18.5
.9	1 to 18.2
.7	1 to 14.7
.6	1 to 12.1
.5	1 to 6.5
.4	1 to 5.25
.2	<sup>a</sup> 1 to 4.0

<sup>a</sup> Obtained from data for 0.2 head.

No attempt was made to determine the exact shape of the sides of the notch. They would be so complex, however, that their construction would render impracticable the use of such notches on the farm. Because of the appreciable difference in the effects of contraction with notches of different sizes, a similar comparison of the discharges through larger notches with those through a 1-foot notch would probably give results different from those obtained for the 2-foot notch.

#### SUBMERGED RECTANGULAR AND CIPOLLETTI NOTCHES

A notch is said to be submerged or "drowned" when the water level on the downstream side is higher than the crest of the notch. To determine the effect of submergence upon the discharges 757 experiments were made with the 1-, 2-, 3-, and 4-foot rectangular and Cipolletti notches used in the free-flow experiments. The conditions on the up-

stream side of the weir were those of the standard weir box—that is, the width of box was 10 feet; the depth of the box 6 feet; and the distance of the floor from the crest of the notches about  $4\frac{1}{2}$  feet. A bulkhead was placed across the escape channel of the standard box, parallel to and about  $5\frac{3}{4}$  feet from the plane of the weir, thus making the spill box 10 feet wide,  $5\frac{3}{4}$  feet long, and 4 feet deep, the floor being about  $2\frac{1}{2}$  feet below the crest of the notch. The height of the water in the escape channel was controlled by a steel head gate 20 inches square with a vertical slide set in the middle of the bulkhead about 0.5 foot above the floor, and by a 4-inch gate valve set near one end of the bulkhead, the finer regulation being made with this valve. The elevation of the water in the escape channel was determined by a hook gauge set in the concrete gauge box, which was connected with the escape channel by two 1-inch pipes which entered near the floor line  $3\frac{1}{2}$  feet from the plane of the weir.

Several minutes were required to adjust the flow of the water before an experiment was started, but when the desired condition of flow had been obtained it was maintained without difficulty throughout the test, except when the head on the upstream side of the weir was high and the head on the downstream side was small. Under this condition the large volume of water flowing through the notch depressed the water surface immediately downstream from the notch. This was followed by a standing wave, and the resulting backlashing and surging in the escape channel caused intermittent pulsations in the hook-gauge still box. The errors, however, were largely compensating, as is indicated by the consistent curves obtained from the experimental data.

The discharges with different heads through the different notches, with free flow and with different depths of submergence, were plotted (figs. 18 to 25) with discharges in cubic feet per second as abscissas and the heads upstream from the weir ( $H_A$ ) as ordinates. Curves were drawn showing the discharges with different heads upstream from the weir ( $H_A$ ) with varying differences ( $H_D$ ) between the head upstream from the weir ( $H_A$ ) and the head downstream from the weir ( $H_B$ ). The method of interpolating between the values given on the curves in figures 18 to 25 is indicated by the dotted lines in figure 18 and is based upon the fact that  $H_A = H_B + H_D$ . The  $H_D = 0.15$  line must pass through the points where the various  $H_B$  lines intersect the  $H_A$  lines and satisfy the equation  $H_A - H_B = 0.15$ . The  $H_B = 0.65$  line would be located similarly upon the points of intersection of the  $H_A$  and  $H_B$  lines. Interpolations for other depths of submergence can be made in the same manner by drawing  $H_A$  lines for other than even 0.05-foot heads. For the purpose of comparison, the free-flow discharge curve is drawn with each set of submergence curves.

A series of experiments was made to determine the effect upon discharges of changing the conditions in the escape channel from free flow

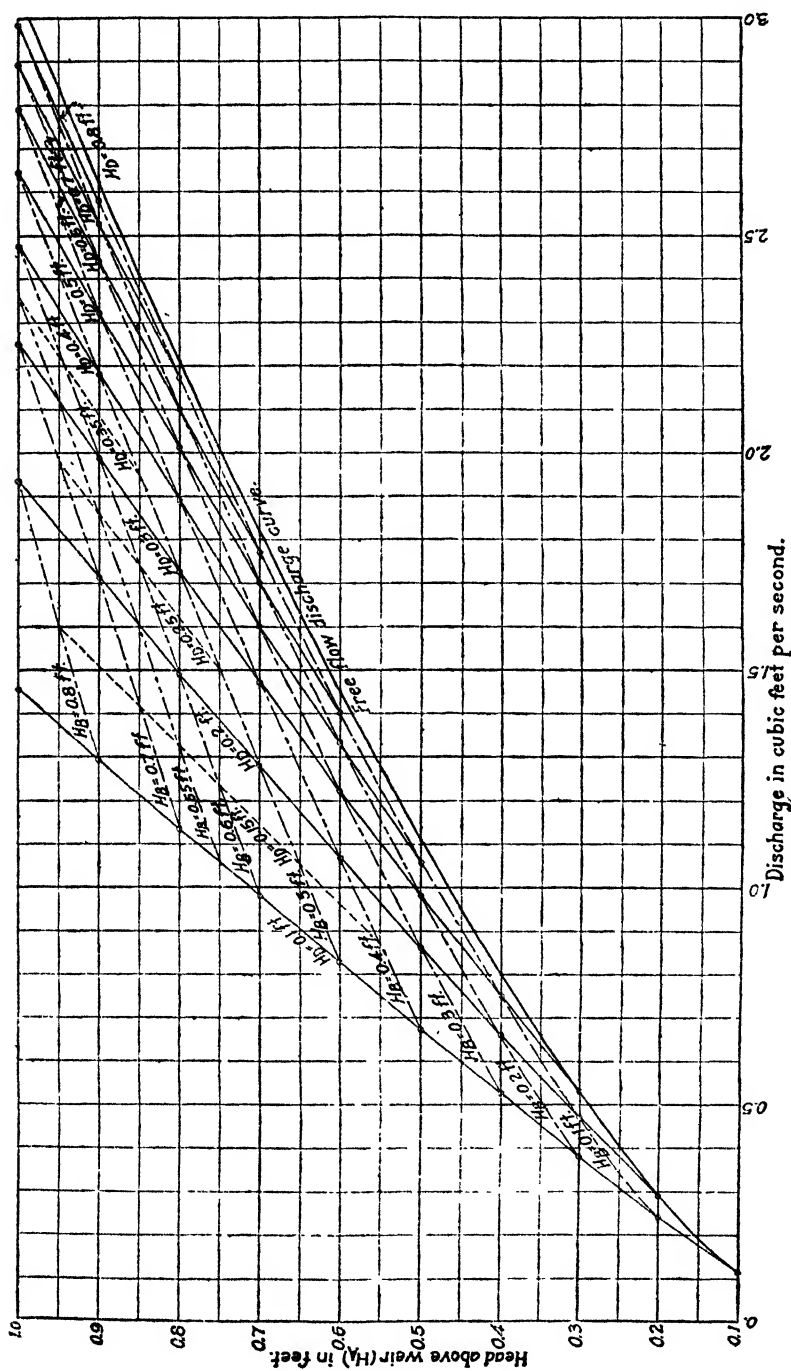


FIG. 18.—Curves showing the discharges through a 1-foot rectangular notch submerged to different depths.  $H_A$  = head above weir;  $H_B$  = head below weir;  $H_B/H_A$  = effective head.



to submergence. In this set of experiments the head upstream from the weir was made constant, but the conditions downstream were changed by stages in the runs from a free fall of 0.5 foot to a submergence of 0.1 foot. The discharges through this change of conditions remained the same within the limit of the experimental error—0.5 per cent. The

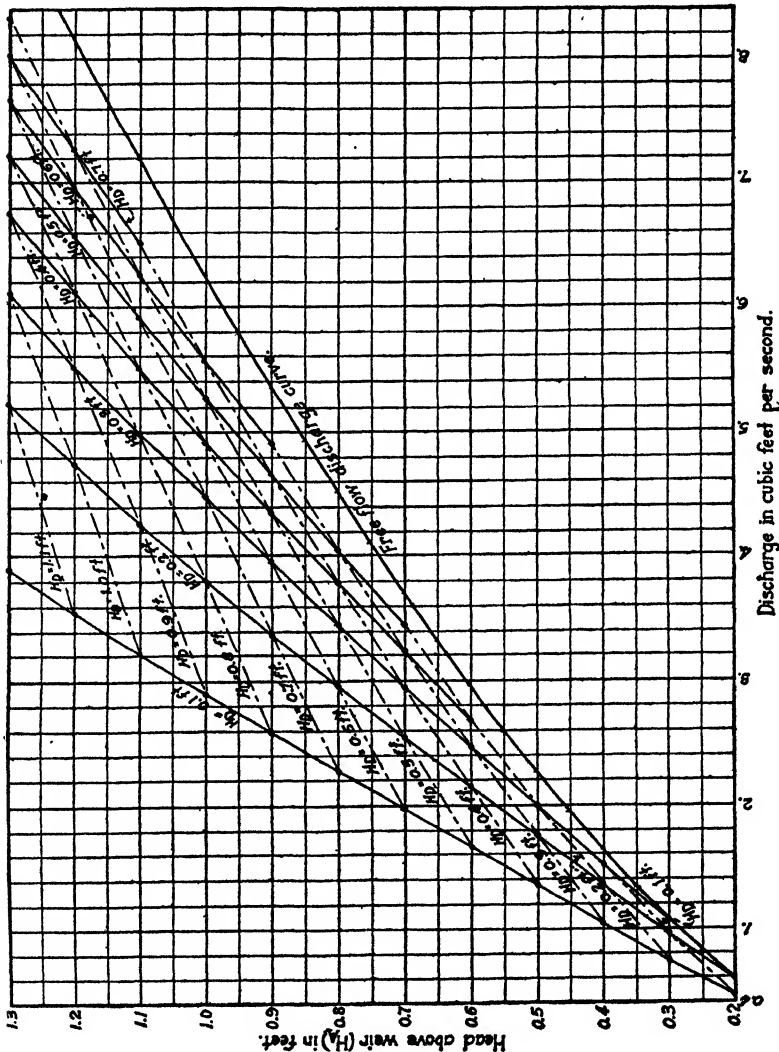


FIG. 19.—Curves showing the discharges through a 2-foot rectangular notch submerged to different depths.  $H_A$ =head above weir;  $H_D$ =head below weir;  $H_D=H_A-H_B$ =effective head.

notches were all thin-edged, the cross section of the weir box in every case was large enough for full-contraction conditions, and the escape channel was wide enough to allow the sheet of water to expand laterally after passing through the notch. In none of the tests was the amount of submergence small enough to make it possible to determine whether the discharge is actually increased with the small amounts of submer-



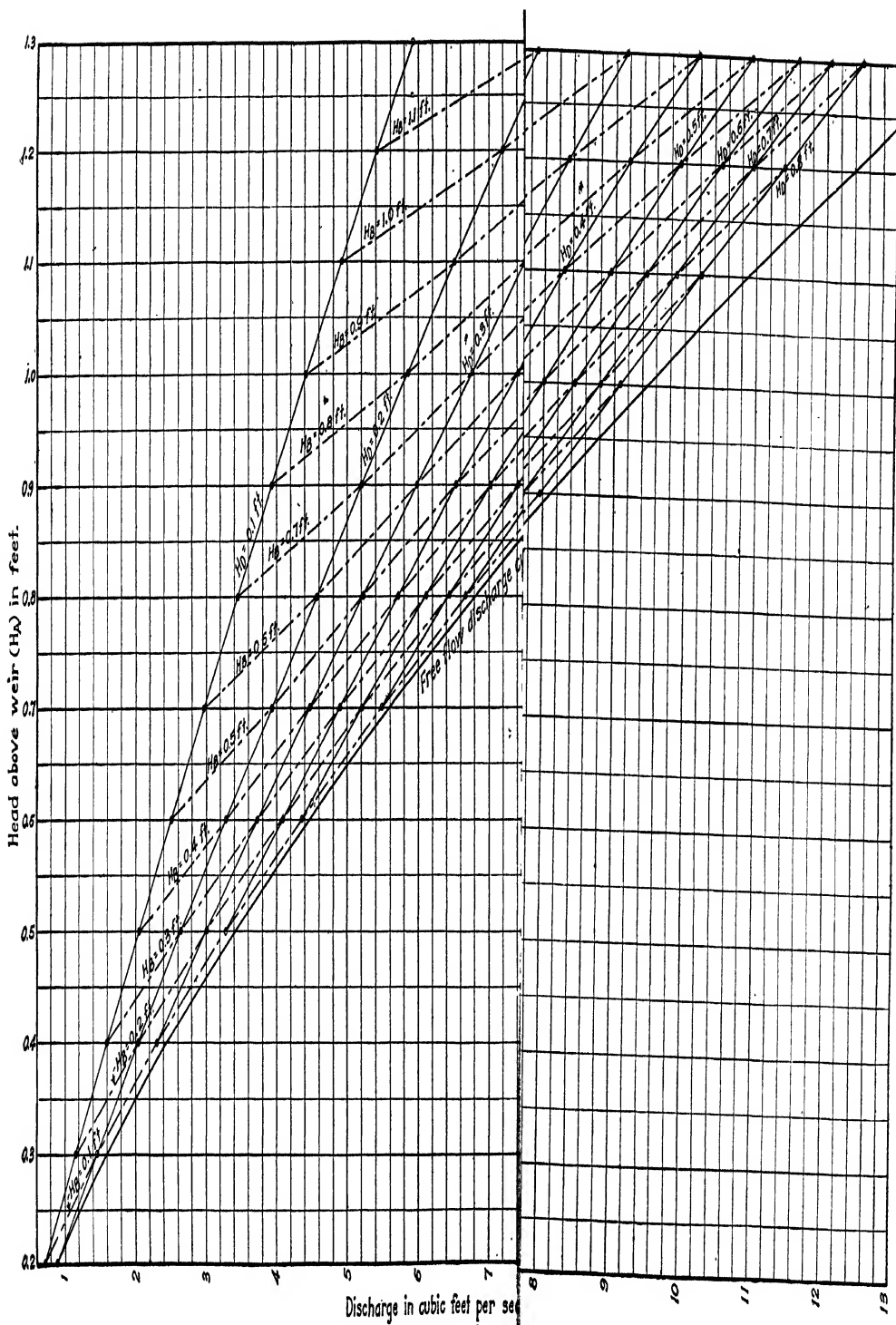


FIG. 20.—Curves showing the discharges through a 3-foot rectangular notch submerged to different depths.

head above weir;  $H_2$ —head below weir;  $H_0 - H_2$ —effective head.



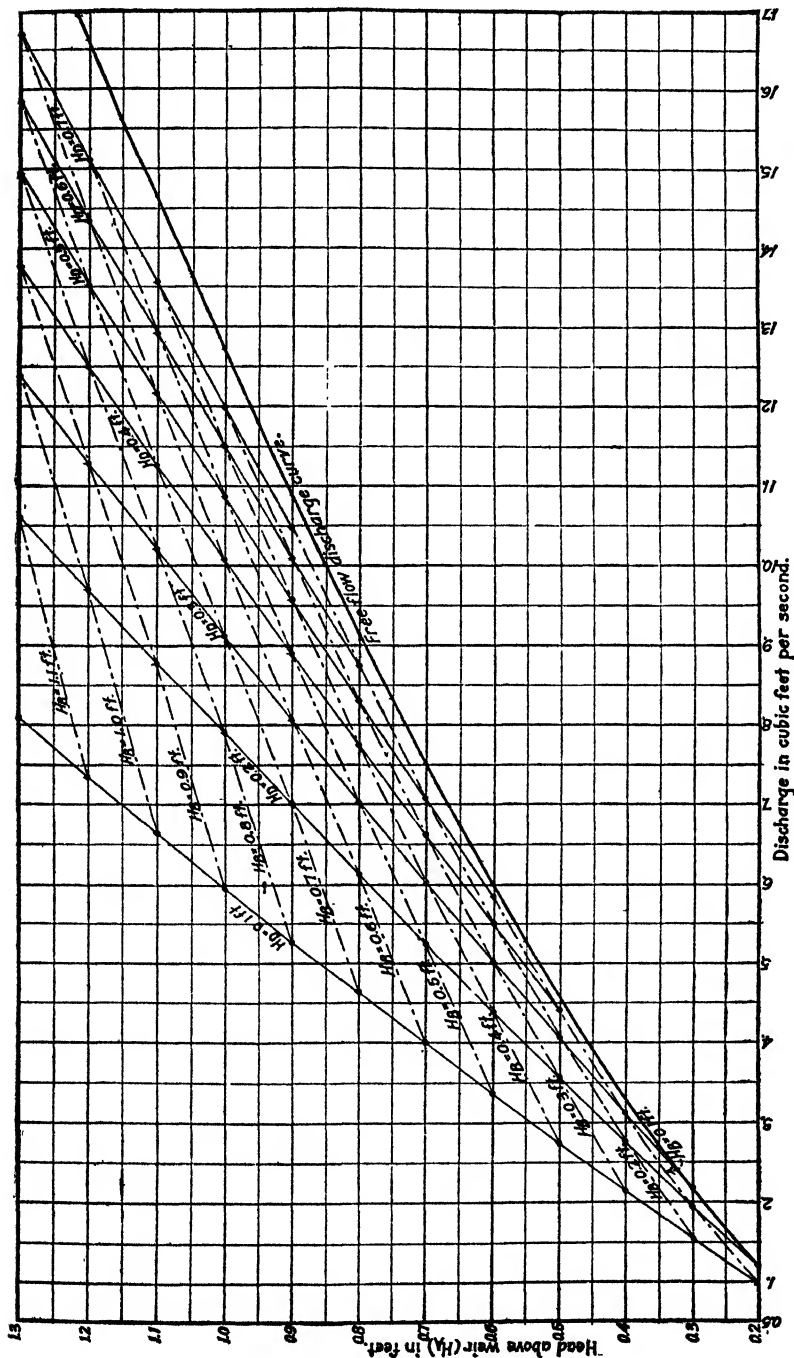


FIG. 21.—Graph showing the discharges through a 4-foot rectangular notch submerged to different depths.  $H_A$ =head above weir;  $H_B$ =head below weir;  $H_D=H_A-H_B$ =effective head.

gence. For all practical purposes, however, it may be stated that the discharge is not materially affected unless the notch is submerged until  $H_B$  is at least one-tenth of  $H_A$ . When  $H_B$  is one-eighth of  $H_A$ , the dis-

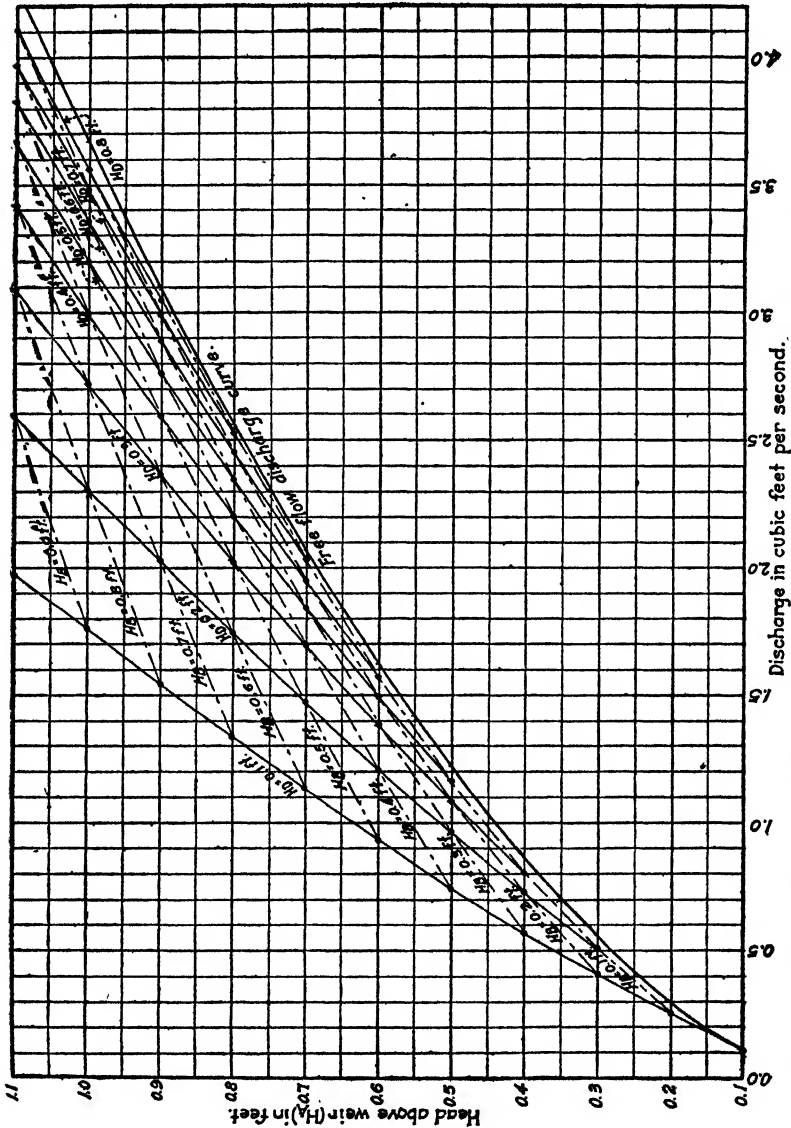


FIG. 22.—Curves showing the discharges through a 1-foot Cipolletti notch submerged to different depths.  $H_A$ =head above weir;  $H_B$ =head below weir;  $H_D=H_A-H_B$ =effective head.

charge is decreased approximately 2 per cent; when it is one-fourth, the decrease is approximately 6 per cent; and when it is one-third, the decrease is approximately 9 per cent. These percentages vary somewhat with the head.

## SUMMARY

(1) The discharges through rectangular and Cipolletti notches when plotted logarithmically do not give straight lines and therefore can not be represented correctly by a formula of the type  $Q = CLH^n$ . It was

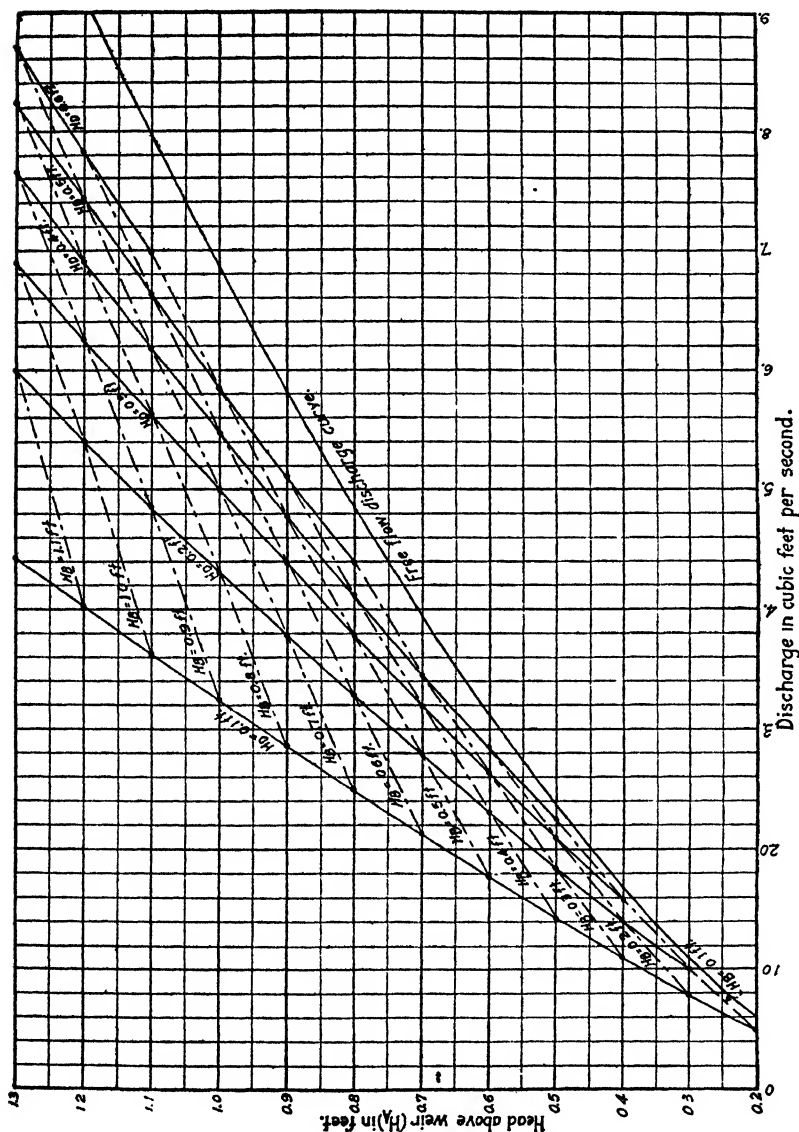


FIG. 23.—Curves showing the discharges through a 2-foot Cipolletti notch submerged to different depths.  $H_A$  = head above weir;  $H_B$  = head below weir;  $H_D = H_A - H_B$  = effective head.

found, however, in the case of the rectangular notches experimented with and the heads of water run, that a straight-line formula could be deduced that within the range of the experiments gave values quite close to the experimental data.

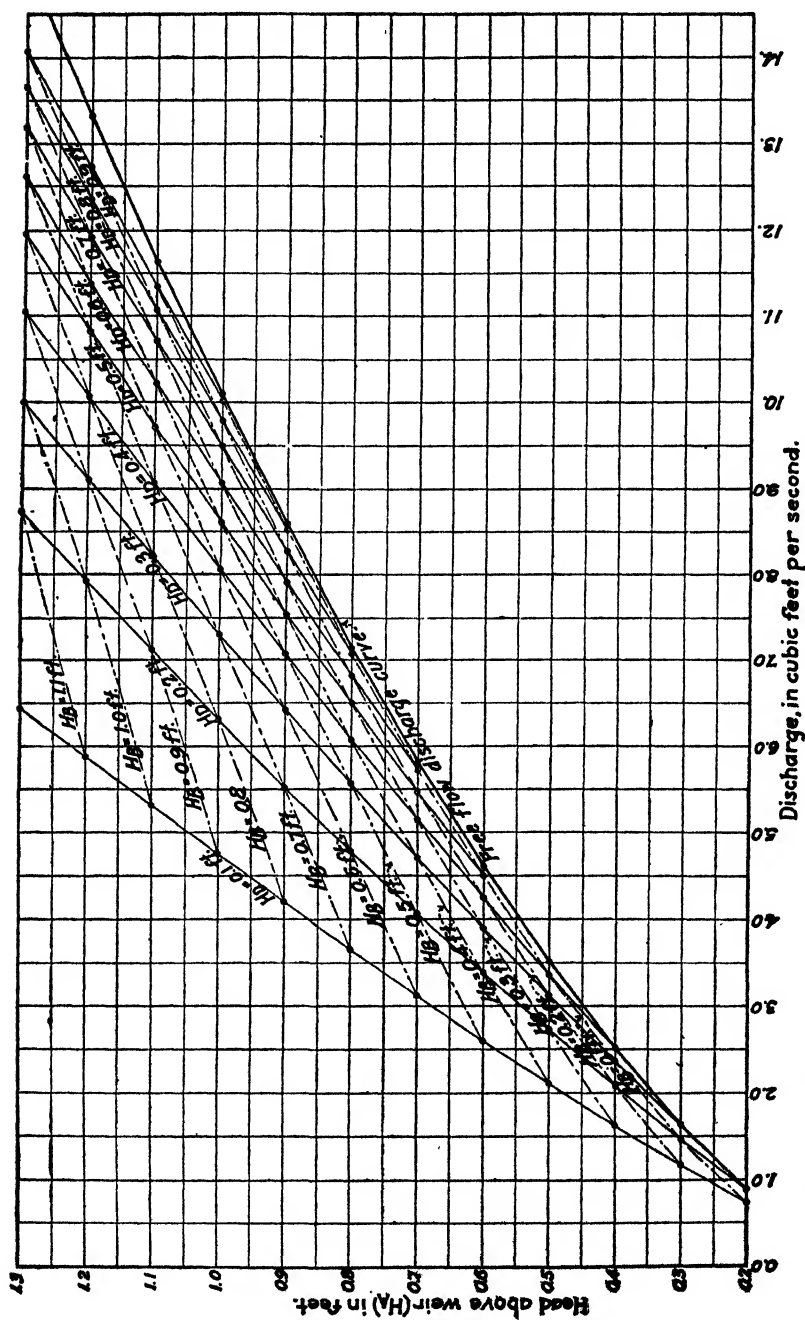


FIG. 24.—Curves showing the discharges through a 3-foot Cipolletti notch weir submerged to different depths.  $H_A$ —head above weir;  $H_B$ —head below weir;  $H_B = H_A - H_B$ —effective head.



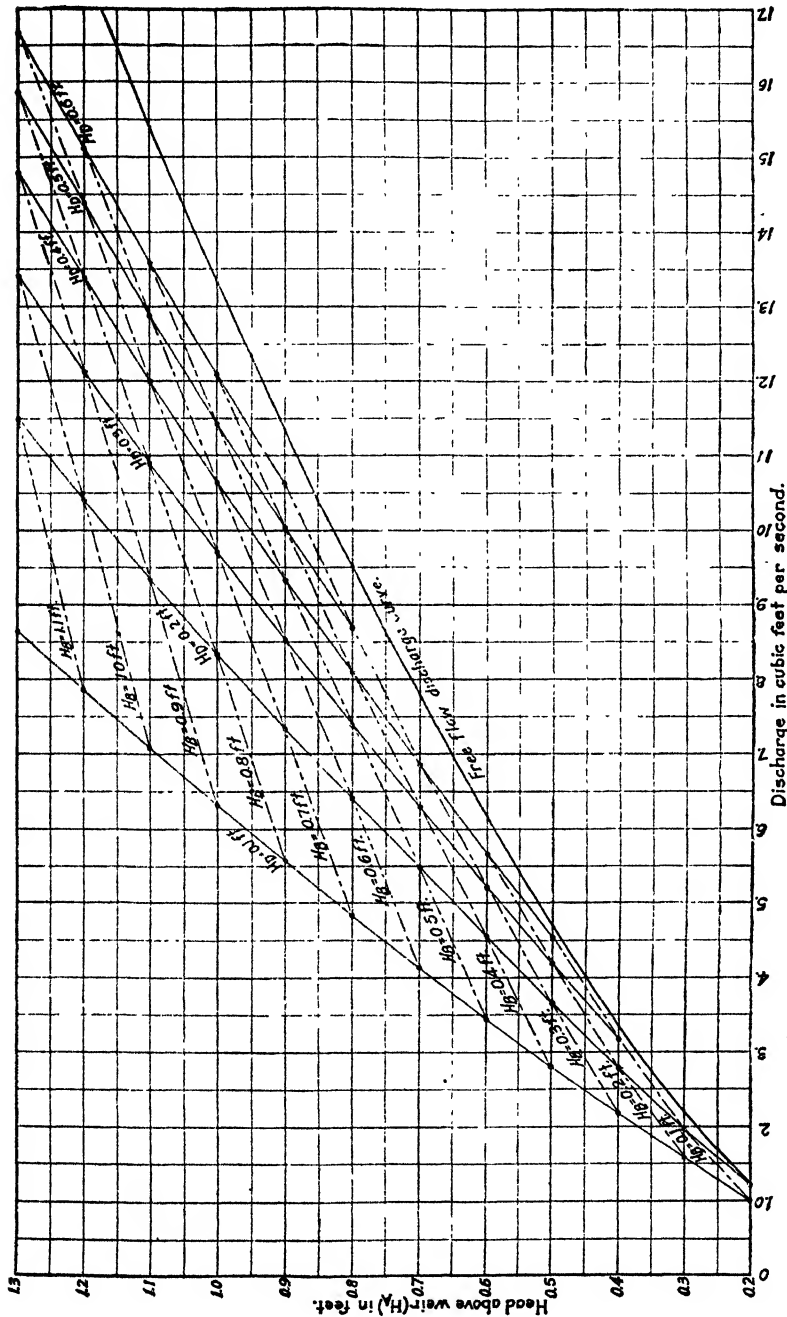


FIG. 25.—Curves showing the discharges through a 4-foot Cipolletti notch submerged to different depths.  $H_A$ =head above weir;  $H_B$ =head below weir;  $H_D=H_A-H_B$ =effective head.

## (2) The formula

$$Q = 3.247LH^{1.48} - \left( \frac{0.566L^{1.8}}{1+2L^{1.8}} \right) H^{1.9}$$

gives discharge values for 1-, 1.5-, 2-, 3-, and 4-foot rectangular notches that agree within a maximum of approximately 1.2 per cent and within an average of 0.28 per cent with the curves plotted from the experimental data.

(3) The discharges through the 0.5-foot rectangular notch do not follow the same law as those for the longer notches. The formula

$$Q = 1.593H^{1.526} \left( 1 + \frac{1}{800H^{2.3}} \right)$$

gives values consistent with the curve plotted from the experimental data.

(4) The Francis formula gives values within approximately 2 per cent of the actual discharges, so long as the head does not exceed one-third the length of the notch.

(5) Within the limits of the experiments the formula

$$Q = 3.08L^{1.022} H^{(1.46+0.008L)}$$

gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot rectangular notches that agree within a maximum of 0.7 per cent, and an average of 0.26 per cent, with the values given in the curves plotted from the experimental data.

(6) The formula  $Q = 1.566H^{1.504}$  gives values for the 0.5-foot rectangular notch that agree within 1 per cent with the curves plotted from the experimental data.

(7) The curve-line formula for rectangular notches takes account of the law of variation of the discharge curves better than does the straight-line formula and, consequently, it appears that it will give closer values for higher heads and longer notches than those experimented with.

(8) The formula

$$Q = 3.247LH^{1.48} - \left( \frac{0.566L^{1.8}}{1+2L^{1.8}} \right) H^{1.9} + 0.609H^{2.5}$$

gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot Cipolletti notches that agree within 0.5 per cent with the curves plotted from the experimental data, except in the case of the lower heads on the 1-foot notch, where the maximum divergence is approximately  $1\frac{1}{2}$  per cent.

(9) The discharges through the 0.5-foot Cipolletti notch do not follow the same law as those for longer notches. The formula

$$Q = 1.593H^{1.526} \left( 1 + \frac{1}{800H^{2.3}} \right) + 0.587H^{2.59}$$

represents the discharges through such a notch.

(10) The Cipolletti formula gives discharge values within  $1\frac{1}{2}$  per cent of the actual discharges so long as the head does not exceed one-third the length of the crest of the notch.

(11) The formula

$$Q = 3.08L^{1.022}H^{(1.46+0.003L)} + 0.6H^{2.6},$$

which is based on the straight-line formula for rectangular notches, gives discharge values for the 1-, 1.5-, 2-, 3-, and 4-foot Cipolletti notches that agree within a maximum of 1 per cent with the curves plotted from the experimental data, the divergences at all but a few points being 0.5 per cent or less. The formula for the 0.5-foot notch is  $Q = 1.566H^{1.504} + 0.56H^{2.55}$ .

(12) The Cipolletti type of notch does not give discharges as nearly proportional to the length of crest as does the rectangular type, consequently, since rectangular notches are simpler to construct and the formula for such notch gives as accurate discharge values as does the formula for Cipolletti notches, the rectangular-notch weir is to be preferred.

(13) The general formula for discharges through triangular notches of from  $28^\circ$   $4'$  to  $90^\circ$ , and probably up to  $109^\circ$ , is

$$Q = (0.025 + 2.462 S)H^{(2.5 - \frac{0.0195}{S^{0.76}})}$$

where  $H$  is the head in feet and  $S$  the slope of the sides. Triangular notches having side slopes greater than about 1 to 4 ( $109^\circ$ ) are impractical, as the nappe adheres.

(14) The  $90^\circ$  triangular notch is the most practical triangular notch and should be used in preference to either rectangular or Cipolletti notches for discharges up to approximately 3 cubic feet per second. The approximate formula  $Q = 2.49H^{2.48}$  will give discharge values for  $90^\circ$  notches which agree very closely with the value obtained with the general formula for triangular notches.

(15) The crest and sides of a weir notch need not be knife-edged. They are sufficiently sharp if the upstream corner of the edges is a distinct angle of  $90^\circ$  or less and the thickness of the edges is not so great that the water will adhere to them.

(16) The head should be measured upstream from the weir a distance of at least  $4H$ , or sidewise from the end of the crest in the plane of weir a distance of at least  $2H$ .

(17) The distances required for full contractions with rectangular and Cipolletti notches are approximately  $2H$ , but an additional cross-sectional area of the weir box is required to reduce the velocity of approach.

(18) With end contractions equal to  $2H$  and a bottom contraction equal to  $3H$ , or end contractions equal to  $3H$  and a bottom contraction equal to  $2H$ , the mean velocities of approach are about  $\frac{1}{3}$  foot

per second, and the discharges with medium to high heads do not agree more closely than approximately 1 per cent with the discharges computed by the formulas.

(19) The average ratio of the cross-sectional area of the weir box ( $A$ ) to the cross-sectional area of the notch ( $a$ ) required to give discharges within 1 per cent of the values obtained with the formula is greater than 7 and is probably near 15.

(20) In order to make the results comparable with those for rectangular notches, the end contractions for trapezoidal notches should be measured from about the middle point of the side of the notch, rather than from the end of the crest.

(21) A notch which would give discharges proportional to the lengths of the notches would probably have curved sides, the slope decreasing with the head.

(22) For all practical purposes, discharges through rectangular and Cipolletti notches are not affected until the notch is submerged to a depth equal to one-tenth the head upstream from the weir. Submergence equal to one-eighth the head upstream from the notch decreases the discharge approximately 2 per cent, that equal to one-fourth approximately 6 per cent, and that equal to one-third approximately 9 per cent.

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# IDENTITY OF ERIOSOMA PYRI

By A. C. BAKER,

*Entomological Assistant, Deciduous Fruit Insect Investigations, Bureau of Entomology*

This paper has been written in order to reinstate the woolly aphid described by Fitch from apple (*Malus* spp.) roots, to point out its distinctness from the woolly apple aphid (*Eriosoma lanigerum* Hausmann), with which it has been confused, and to place it among the species of the genus to which it properly belongs.

In 1851 Fitch<sup>1</sup> described a woolly aphid under the name "*Eriosoma pyri*." At the same time he described the work of what seems to be *E. lanigerum* Hausmann on apple. At the time of his original description Fitch evidently did not know of the genus Pemphigus. This is indicated from his remarks in his first report,<sup>2</sup> for in the description in this publication he is quite positive in placing his species in that genus. The description of the wingless forms agrees well, however, with *lanigerum*.

The identity of *pyri* has for many years been in doubt, and the name has been referred to different species as a synonym. The writer,<sup>3</sup> in his recent work on the woolly aphid, considered it to be *lanigerum*. This was based on two things: The description of the wingless forms, with the possibility of abnormality in the winged form, and Gillette's<sup>4</sup> statement in regard to the type. One fact, however, seems evident. The descriptions given by Fitch for his winged forms could not have been made from normal migrants of *lanigerum*. In fact, they could not have been made from winged forms of *lanigerum* at all. This is particularly true of the description in the first report.

Fitch's original notes on the species are now in the writer's hands, and they throw some interesting light on the question. After describing the wings minutely, Fitch says: "The wings serve best to distinguish this species, and an exact figure of one or both of them will be the best illustration of it that can be given," and again, "Neuration of the wings identical with that of *Myzoxylus imbricator*." By 1871 Fitch had some feeling that his *pyri* might be a synonym of *lanigerum*, for in his notebook, under October 11 of that year, he suggests such a possibility. He adds, "My winged *lanigera* from Dr. Signoret is a Pemphigus, the 3rd vein being simple, but not so abortive at its base, and has all the veins slender."

<sup>1</sup> Fitch, Asa. Catalogue with references and descriptions of the insects collected and arranged for the State Cabinet of Natural History. In 4th Ann. Rpt. [N. Y.] State Cab. Nat. Hist., p. 68. 1851.

<sup>2</sup> —[Report on the Nuxious and Other Insects of the State of New York.] p. 7. In Trans. N. Y. State Agr. Soc., v. 14, 1854, p. 711. 1855. Reprint, p. 7, Albany, N. Y., 1856.

<sup>3</sup> Baker, A. C. The woolly apple aphid. U. S. Dept. Agr. Office Sec. Rept. 101, p. 13. 1915.

<sup>4</sup> Gillette, C. P. Plant louse notes, family Aphididae. In Jour. Econ. Ent., v. 2, no. 5, p. 352. 1909.

This much remains: Fitch was not sure that he was not dealing with a compound species in his apple-root form and his winged forms. This is shown by the following note: "Amyot describes *Eriosoma lanigerum* as producing excrescences. Can these small lice be that species, and the winged ones another species accidentally present with them?"

What Fitch suspected is, the writer believes, true, and Fitch described the winged form of one species and the work of wingless *lanigerum*.

In the United States National Museum collection there is some material labeled "*P. pyri* Fitch, Type," and mounted by Pergande from the Fitch collection. This proves to agree in every detail with the different descriptions of the winged forms given by Fitch. There seems good reason to believe that the material represents the specimens from which Fitch drew up his diagnosis. This is strengthened by the fact that the species occurs in the vicinity of Washington, D. C., and Vienna, Va., upon apple and upon pear (*Pyrus* spp.) roots. It is particularly common upon pear roots, and it occurs also upon *Crataegus* spp. and ash (*Fraxinus* spp.).

Since this material seems to settle finally the standing of *pyri*, a description is here given of the form based upon this material and upon other specimens collected mostly from pear roots. The form proves to belong to the genus *Prociphilus*, and in order to separate it from other species of the genus, descriptive notes and figures are given of the other species known to the writer. Particular stress is laid in these notes on the dorsal wax plates of the thorax, since these seem to prove good diagnostic characters.

The writer has never seen specimens of *Prociphilus crataegi* Tullgren, and it may be possible that *pyri* and *crataegi* are the same, since the sensory characters are similar. There seems, however, to be considerable difference in measurements. The question as to their distinctness or identity can only be determined by a careful comparison of the two.

It is possible, also, that *venafuscus* Patch may prove to be *pyri*. But in the specimens studied by the writer the sensoria are much more even, and *pyri* seems to lack the small, pointed projection near the base of the third segment of the antennæ.

The following description will, however, serve to place *pyri*:

#### *Prociphilus pyri* (Fitch)

Fall migrant (fig. 1, E, Q).—Morphological characters: Antennal segments as follows: I, 0.064 mm.; II, 0.096 mm.; III, 0.544 mm.; IV, 0.224 mm.; V, 0.24 mm.; VI, base 0.192 mm., unguis 0.064 mm.; segments III to VI with transverse sensoria, usually very irregular in disposition and giving the segments, particularly segment III, a gnarled appearance; segment III with 28 to 35 sensoria, segment IV with 8 or 9, segment V with about the same number, and segment VI with 3 to 6. These sensoria are on the underside of the antennæ, the upper surface being armed with a few hairs situated on tubercles. Head above with two oval or almost circular transparent wax plates. Dorsum of thorax with a pair of rather small, somewhat triangular wax plates. Forewings 4.38 mm. long and 1.43 mm. wide at their greatest width. Hind tibiæ 1.2 mm. long. Length from vertex to tip of cauda, 2.48 mm.



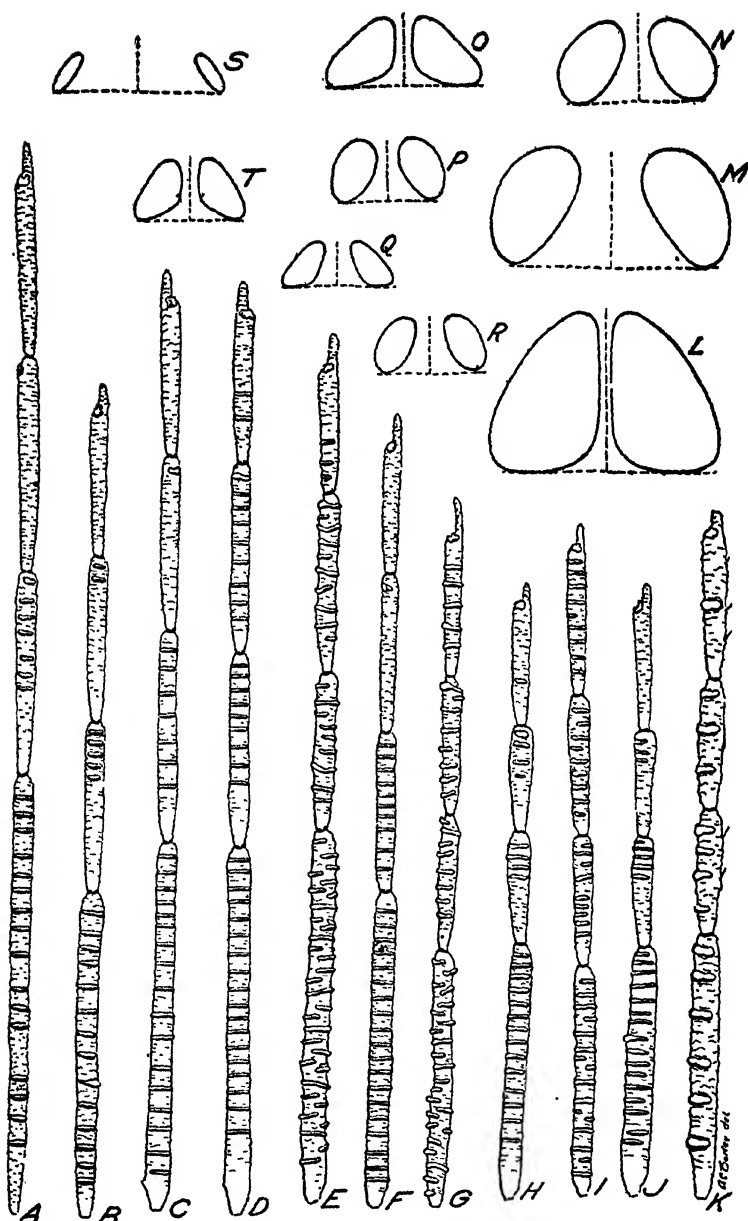


Fig. 1.—Structural characters of the species of *Prociphilus*. A, *P. bumulae*: Distal segments of antenna of spring migrant. B, *P. poschingeri*: Distal segments of antenna of spring migrant. C, *P. venafuscus*: Distal segments of antenna of spring migrant. D, *P. venafuscus*: Distal segments of antenna of fall migrant. E, *P. pyri*: Distal segments of antenna of fall migrant. F, *P. xylostei*: Distal segments of antenna of spring migrant. G, *P. populi-conduplicifolius*: Distal segments of antenna. H, *P. corrugatus*: Distal segments of antenna of spring migrant. I, *P. corrugatus*: Distal segments of antenna of spring migrant. J, *P. alni-foliae*: Distal segments of antenna. K, *P. tessellatus*: Distal segments of antenna. L, *P. bumulae*: Thoracic wax plates. M, *P. poschingeri*: Thoracic wax plates. N, *P. xylostei*: Thoracic wax plates. O, *P. venafuscus*: Thoracic wax plates. P, *P. corrugatus*: Thoracic wax plates. Q, *P. pyri*: Thoracic wax plates. R, *P. alni-foliae*: Thoracic wax plates. S, *P. populi-conduplicifolius*: Thoracic wax plates. T, *P. tessellatus*: Thoracic wax plates.

Color characters: Eyes, antennæ, and legs black; head black; prothorax and abdomen dull olive green with darker green marginal patches on the abdomen. Thoracic lobes and sternal plate black. Wing veins dark, with dusky bordering; the entire wing often more or less smoky. Head and thorax with a bluish white bloom; abdomen with a long cottony secretion, most pronounced caudad.

**Prociphilus aceris** (Monell).

Specimens of this species have a pair of large circular wax plates upon the head, and the dorsal wax plates of the thorax are of the same size and shape as those of *venafuscus* Patch. The sensoria on the third segment of the antennæ are oval in shape, some almost circular. They are thus not typical for the genus, but approach those of *attenuatus* Osborn and Sirrine for which Dr. E. M. Patch, of the Maine Experiment Station, has erected the genus *Neoprociphilus*. There seems to be, however, a gradual gradation from the type to this species. The wing also suggests that of *attenuatus*, and there is some doubt in the writer's mind in regard to the distinctness of *Neoprociphilus*. The measurements of antennal segments are as follows: III, 0.416 mm.; IV, 0.256 mm.; V, 0.24 mm.; VI, base 0.272 mm., unguis 0.048 mm.

**Prociphilus alnifoliae** (Williams) (fig. 1, J, R).

*Alnifoliae* is a species of medium size with rather short antennæ. The sensoria do not, as a rule, extend entirely across the segments, and they are often acute at each end, thus touching the margins of the segments as a point. The dorsal wax plates of the thorax are quite similar to those of *corrugatus*, being small and oval.

**Prociphilus bumulae** (Schrank) (fig. 1, A, L).

This species is very large and the sensoria of the antennæ are even and do not usually extend beyond the margins of the segment. The dorsal wax plates of the thorax are large and triangular and situated close together. In some specimens they almost touch along the median line. The measurements of antennal segments are as follows: III, 0.704 mm.; IV, 0.32 mm.; V, 0.32 mm.; VI, base 0.288 mm., unguis 0.064 mm.

**Prociphilus corrugatus** (Sirrine) (fig. 1, H, I, P).

This insect is a rather small species with regular sensoria present on the antennæ of the spring migrant, but with them irregularly arranged on the antennæ of the fall migrant. The dorsal wax plates of the thorax are small and oval in outline. The measurements of the antennal segments are: III, 0.32 mm.; IV, 0.144 mm.; V, 0.16 mm.; VI, base 0.128 mm., unguis 0.032 mm.

**Prociphilus fraxini-depetalae** (Essig).

This species appears to be a synonym of *venafuscus* Patch.

**Prociphilus imbricator** (Fitch).

This well-known species has not been figured. The sensoria of the antennæ are rather large, approaching those of *tessellatus* (Fitch). The dorsal wax plates of the thorax are small and well separated. The measurements of antennal segments are as follows: III, 0.368 mm.; IV, 0.176 mm.; V, 0.176 mm.; VI, base 0.192 mm., unguis 0.048 mm.

**Prociphilus populiconduplifolius** (Cowen) (fig. 1, G, S).

The antennæ of this species are characteristic in that the sensoria extend past the edges of the segments and give them an irregular or beaded effect on the margins. The wax plates on the thorax are also very characteristic, being minute and very widely separated. The antennal measurements are as follows: III, 0.4 mm.; IV, 0.288 mm.; V, 0.208 mm.; VI, base 0.208 mm., unguis 0.064 mm.

In the writer's opinion there is not sufficient difference for the retention of the genus *Thecabius*. The habits of the stem mothers may be different, as indicated by *patchii* Gillette, and yet the insects are very close in structure. The wax plates and sensoria vary greatly within the genus.

***Prociphilus poschingeri* (Holzner) (fig. 1, B, M).**

Placed usually as a synonym of *bumulae* Schrank, this form as represented by our specimens shows some differences. The insects are considerably smaller and the dorsal wax plates of the thorax are not triangular and close together as are those of *bumulae*, but are considerably separated and oval in outline. Measurements of antennal segments: III, 0.496 mm.; IV, 0.246 mm.; V, 0.246 mm.; VI, base 0.224 mm., unguis 0.048 mm.

***Prociphilus tessellatus* (Fitch) (fig. 1, K, T).**

The antennae of *tessellatus* are hardly typical for this genus. The species seems, however, to fit here as well as anywhere. The sensoria on the antennae are very broad for the genus and the shape of the segments is not typical. The dorsal wax pores are, however, quite normal. They are somewhat triangular in shape and are somewhat smaller than those of *venafuscus*. In many specimens each is armed with a small hair. Measurements of antennal segments: III, 0.4 mm.; IV, 0.171 mm.; V, 0.171 mm.; VI, base 0.197 mm., unguis 0.032 mm.

***Prociphilus venafuscus* (Patch) (fig. 1, C, D, O).**

The form described by Dr. Patch<sup>1</sup> is the most typical American species and the antennal characters are very similar to those of *bumulae* Schrank. The clouding of the wings met with in *venafuscus* is present also in our specimens of *poschingeri* though it is not noted in those of *bumulae*. The dorsal wax plates of the thorax are, in *venafuscus*, triangular like those of *bumulae*. They are, however, very much smaller. Measurements of antennal segments: III, 0.56 mm.; IV, 0.288 mm.; V, 0.288 mm.; VI, base 0.224 mm., unguis 0.049 mm.

***Prociphilus xylostei* (De Geer) (fig. 1, F, N).**

Specimens of this species are much smaller than those of *bumulae* or even those of *venafuscus*. The antennal characters are very similar to those of *venafuscus*. The dorsal wax plates of the thorax are, however, of quite different shape in the two species, although they are almost equal in size. Measurements of antennal segments: III, 0.48 mm.; IV, 0.24 mm.; V, 0.24 mm.; VI, base 0.197 mm., unguis 0.048 mm.

The average number of sensoria on the antennae of the species figured is shown in the illustration. The number varies somewhat in different individuals

<sup>1</sup> Patch, Edith M. Aphid pests of Maine. In Maine Agr. Exp. Sta. Bul. 202, p. 174. 1912.

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### A NEW PENETRATION NEEDLE FOR USE IN TESTING BITUMINOUS MATERIALS

By CHARLES S. REEVE, *Chemist*, and FRED P. PRITCHARD, *Assistant Chemist, Office of  
Public Roads and Rural Engineering*

During the early period of the bituminous paving industry the asphaltic cement was usually tested by chewing a small piece and judging its consistency by its resistance to the teeth. With the development of the industry and specifications for work of this character it soon became evident that some more definite method of determining and defining consistency must be evolved, and in 1889 H. C. Bowen, of Columbia University, first described<sup>1</sup> a machine for the purpose. This was followed some years later by the machines designed by A. W. Dow<sup>2</sup> and by Richardson and Forrest.<sup>3</sup>

All of these machines had for their basic principle the depth to which a No. 2 sewing needle would penetrate the material under certain specified conditions of load, time, and temperature. Most, if not all, needle manufacturers produce No. 2 sewing needles, all makes of which are not necessarily of the same shape and size. Since it has, however, been generally understood that the No. 2 needle manufactured by R. J. Roberts was that most often used for the selection of standard needles, the subcommittee of the American Society for Testing Materials which has the penetration test under investigation made the following recommendation in 1915:<sup>4</sup>

The needles for this test shall be R. J. Robert's Parabola Sharps No. 2. They shall be carefully selected by the use of a hand glass, rejecting all that are manifestly of unusual shape or taper. Needles thus selected shall be compared with a standard

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<sup>1</sup> Bowen, H. C. An apparatus for determining the relative degree of cohesion of a semi-liquid body. *In* School Mines Quart., v. 10, no. 4, p. 297-302, 2 fig. 1889.

<sup>2</sup> Dow, A. W. The testing of bitumens for paving purposes. *In* Amer. Soc. Testing Materials, Proc. 6th Ann. Meeting, 1903, v. 3, p. 354. 1903.

<sup>3</sup> Richardson, Clifford, and Forrest, C. N. The development of the penetrometer as used in the determination of the consistency of semi-solid bitumens. *In* Amer. Soc. Testing Materials, Proc. 10th Ann. Meeting, 1907, v. 7, p. 626-631, 3 fig. 1907. Discussion, p. 632-637.

<sup>4</sup> Report of sub-committee on penetration. *In* Amer. Soc. Testing Materials, Proc. 18th Ann. Meeting, 1915, v. 15, pt. 1, p. 353. 1915.

needle and further rejections made of those which vary more than one point from that obtained with the standard needle, on a sample having a penetration of approximately 60.

The committee further stated that it did not think it advisable to recommend at the present time a standard needle for reference, deferring such action until the next annual meeting of the society. Until such recommendation is made needles furnished with penetration machines are to be considered standard.

The above-recommended practice is representative of the method for selecting needles which has been followed in the Office of Public Roads and Rural Engineering, and the standard used for comparison and selection was a needle originally supplied with the penetration machine in use. It has been, however, not uncommon practice in certain laboratories to purchase a package of No. 2 needles and to use them on the assumption that they possessed the requisite dimensions and shape. In an effort to prove the fallacy of such an assumption the authors have taken an enlarged photograph of a package of Roberts's No. 2 needles, an examination of which will serve to make clear the ordinary variations in point, shape, and taper (Pl. LXXXII, fig. 1).

These variations are more clearly shown through a consideration of the results obtained in selecting needles to be used for routine testing in the office. Several packages were first sorted with the aid of a magnifying glass and micrometer caliper, and a selection made of those whose shape and size appeared to be identical with the shape and size of the standard. From a lot of 72 needles, only 12 were thus selected. From these 12, those were selected for use which gave practically identical results in the penetrometer with a so-called standard needle on two samples of oil asphalt. The results of these tests are given in Table I, from which it may be seen that only 5 of the 12 needles fulfilled the requirements. Needles that failed to give accepted values on the harder materials were not tried on the softer.

Inasmuch as only 5 needles out of 72 proved acceptable, it may be seen what results would follow from the indiscriminate use of No. 2 needles as such.

It is further to be noted particularly that there is no existing single standard with which comparison can be made, owing to the fact that there is no means of accurately defining or gauging the type of needle in use. The work herein described was undertaken for the purpose of devising, if possible, a needle which would give results practically identical with results now obtained in using the so-called standard needles, and which could be accurately described and duplicated at any time.

The standard needle on file in the office is 1.8 inches in length, with a diameter of 0.040 inch for a length of 1 inch from the eye. The remainder of the needle tapers in a parabolic curve to a sharp point. The simpler

needle to define would be one having a straight taper. Round, polished, annealed-steel drill rods having diameters of 0.042 inch were therefore cut into 2-inch lengths and pointed at one end with tapers having a length of  $\frac{1}{8}$ ,  $\frac{1}{4}$ ,  $\frac{5}{8}$ , and  $\frac{3}{8}$  inch. Each needle was tempered and highly polished, then tested in the penetrometer on a material showing a penetration of 140 with the standard needle. The penetrations were as follows on needles made from 0.042-inch drill rod:  $\frac{1}{8}$ -inch taper, 125;  $\frac{1}{4}$ -inch taper, 127;  $\frac{5}{8}$ -inch taper, 129;  $\frac{3}{8}$ -inch taper, 134.

TABLE I.—Results of a standardization test of penetration needles on oil asphalt

[Accepted values 6.8, 6.9, 7.0]

Needle No.	Oil asphalt 1					Oil asphalt 2.	
	Operator C.	Operator F.	Operator D.	Operator A.	Operator E.	Operator C.	Operator F.
Standard . . . . .	6.9	6.9	6.9	7.0	7.0	{ 15.9 15.6	15.7 15.6
1 (rejected) . . . . .	7.2	7.3	.....	.....	.....	.....	.....
2 (O. K.) . . . . .	6.8	6.6	.....	.....	{ 6.8 6.9	15.9	15.9
3 (O. K.) . . . . .	6.9	6.7	.....	6.85	.....	15.8	15.8
4 (rejected) . . . . .	7.1	7.4	.....	.....	.....	.....	.....
5 (rejected) . . . . .	7.1	7.0	.....	.....	7.1	.....	.....
6 (O. K.) . . . . .	{ 6.7 6.7	{ 6.95 6.95	{ 6.8	6.95	.....	15.9	15.9
7 (rejected) . . . . .	6.8	6.6	.....	{ 6.55 6.3	.....	.....	.....
8 (rejected) . . . . .	6.9	6.5	.....	.....	6.7	.....	.....
9 (rejected) . . . . .	{ 6.8 6.7	{ 6.6	.....	6.55	.....	.....	.....
10 (O. K.) . . . . .	6.9	{ 6.75 6.75	.....	.....	6.8	16.0	16.0
11 (O. K.) . . . . .	6.9	6.85	.....	.....	.....	{ 15.9 16.0	16.0
12 (rejected) . . . . .	6.8	6.6	.....	.....	{ 6.4 6.75	.....	.....

While none of these needles yielded as high results as the standard, the one showing the highest values was tested on a sample of material having a penetration of 95 with the standard needle. A penetration of 103 was obtained. This eliminated the 0.042-inch drill rod from further consideration, since it was evident that a taper which would check with the standard needle on softer materials would give higher results than the standard on harder materials.

Drill rod with a diameter of 0.041 inch was then tried. This actually measured 0.0405 inch, and the finished and polished needle from it had a diameter of 0.040 inch. Three pieces of that diameter were given tapers of  $\frac{1}{8}$ ,  $\frac{1}{4}$ , and  $\frac{5}{8}$  inch, respectively, then polished and tested in comparison with the standard needle. The results on four samples of bituminous materials are given in Table II.

TABLE II.—Results of an asphalt penetration test with a needle made from a steel drill rod 0.041 inch in diameter

Taper of needles.	Sample No. 5284 (blown oil asphalt).	Sample No. 5963 (oil asphalt).	Sample No. 5985 (blown oil asphalt).	Sample No. 8928 (fluxed native asphalt).
Standard.....	30	153	75	109
$\frac{1}{16}$ -inch taper.....	30	148	70	106
$\frac{1}{4}$ -inch taper.....	32	150	74	109
$\frac{1}{8}$ -inch taper.....	34	153	80	112

It will be noted from the above that on all four samples, representing three different types of material, the needle with  $\frac{1}{4}$ -inch taper gave results in comparatively closer accord with those obtained by the standard needle than did the others. Three new needles of this type were therefore made and tested in comparison with the standard needle on various types of bituminous material having a wide range of penetration. The results are given in Table III. When it was found that all three needles checked with the standard throughout, the No. 1 new needle was run comparatively with the standard on six additional products, covering a still wider range of materials, in order to determine whether products varying in their general adhesive character might have any effect on the results. It will be noted by referring to Table III that the needle which the writers have designed yields in all cases results practically identical with those obtained with the standard needle. In cases where no results are given for the No. 3 needle the omission is due to the fact that the samples were run before the third needle had been prepared. In all cases but one the results are given by two operators.

TABLE III.—Results of a comparative test of the new penetration needle with a standard needle

Sample No.	Material.	Standard needle.		Needle No. 1.		Needle No. 2.		Needle No. 3.	
		Operator A.	Operator B.	Operator A.	Operator B.	Operator A.	Operator B.	Operator A.	Operator B.
5959	Blown Texas oil asphalt.....	8	8	9	9	8	8	.....	.....
5284	do.....	30	31	32	32	32	32	.....	.....
8233	Mexican oil asphalt.....	41.5	41.5	43	41.5	41	41	.....	.....
8061	California oil asphalt.....	49	47	47	48	48	49	49	47
6811	Texas oil asphalt.....	77	76	77	78	76	76	.....	.....
8916	do.....	93	92	95	95	93	93	.....	.....
8962	California oil asphalt.....	94	94	94	94	93	93	94	94
6291	Texas oil asphalt.....	108	110	110	110	109	108	.....	.....
5406	Oil asphalt (cut-back).....	114	114	111	111	114	114	113	112
8966	Mexican oil asphalt.....	118	121	118	120	117	117	117	119
8970	do.....	119	118	117	119	119	119	118	119
8963	California oil asphalt.....	135	133	134	133	135	133	135	136
5381	Oil asphalt (cut-back).....	133	134	135	135	135	135	134	135
5963	Texas oil asphalt.....	151.5	150	150	151	151.5	151	.....	.....
5559	Oil asphalt (cut-back).....	168	170	170	170	170	168	170	169
8964A	Fluxed California asphalt.....	192	193	192	194	195	196	195	193
8963B	do.....	236	239	235	236	234	238	233	234
8963C	do.....	292	295	295	.....	295	.....	291	.....
5118	Fluxed Trinidad asphalt.....	83	85	83	85	.....	.....	.....	.....
5119	Fluxed Cuban asphalt.....	65	65	65	67	.....	.....	.....	.....
8226	Fluxed Bermudez asphalt.....	45	46	46	46	.....	.....	.....	.....
8015	do.....	115	115	113	115	.....	.....	.....	.....
6293	Blown Gilsonite oil asphalt.....	140	140	138	138	.....	.....	.....	.....
5104	do.....	60	60	59	60	.....	.....	.....	.....



About the time this work was completed, a second standard needle was obtained from the same source as the one used in the foregoing tests. In order to determine the accuracy with which a number of the new type of needle could be readily made, seven were prepared and checked against both the old and new standard on two distinct types of bituminous material. The results are given in Table IV. Each result is an average of at least three determinations.

TABLE IV.—Results of a comparative test of new and old standard penetration needles and seven others of the new type

Needle.	Sample No. 8957 (Gilsonite blown oil asphalt).	Sample No. 8962 (California asphalt).	Needle.	Sample No. 8957 (Gilsonite blown oil asphalt).	Sample No. 8962 (California asphalt).
New standard . . . .	94	96	Needle No. 4. . . . .	92	96
Old standard . . . .	91	96	Needle No. 5. . . . .	91	96
Needle No. 1. . . . .	89	94	Needle No. 6. . . . .	91	96
Needle No. 2. . . . .	90	97	Needle No. 7. . . . .	90	95
Needle No. 3. . . . .	91	95			

It will be noted from the above that all seven new needles check very closely with the old standard needle on both samples, and that on sample 8957 they check closer with the old standard than do the two standards with one another. The lack of uniformity in the shape of the two standard needles, the uniformity of the new type of needle, and the relative shapes of the old and new forms of needle are shown in Plate LXXXIII, figure 2, which is a reproduction of an enlarged photograph of the two standard and seven new needles referred to in Table IV.

The following conclusions are offered as a result of the above investigation:

(1) That the No. 2 sewing needle which has heretofore been used for the penetration test can not be taken indiscriminately, but must be carefully selected and standardized.

(2) That there is no recognized established standard with which new needles can be compared, and that it is not feasible to accurately describe the dimensions of a parabola needle.

(3) That the so-called standard needles furnished with penetration machines may vary among themselves.

(4) That the writers have designed a needle which gives results in close accord with existing standards and has, moreover, the advantage of being accurately described and easily reproduced.

(5) The needle is made by placing a 2-inch length of 0.041-inch annealed-steel drill rod in the chuck of a high-speed lathe, and by means of a fine sharp file turning the end to a sharp point having a  $\frac{1}{4}$ -inch taper. When it has been made as smooth and sharp as possible by this means,

the needle is tempered,<sup>1</sup> then ground to a sharp point with a good stone, after which it is smoothed and polished with emery dust, crocus cloth, and rouge, and finally held carefully on a buffing wheel. The finished needle should be sufficiently smooth and sharp to enter and pass through a piece of ordinary writing paper without sticking or friction. In other words, this new needle must have as sharp a point and smooth a surface as any sewing needle. The important thing is to have the taper straight, beginning  $\frac{1}{4}$  inch from the end, and the needle above the taper exactly 0.04 inch in diameter.

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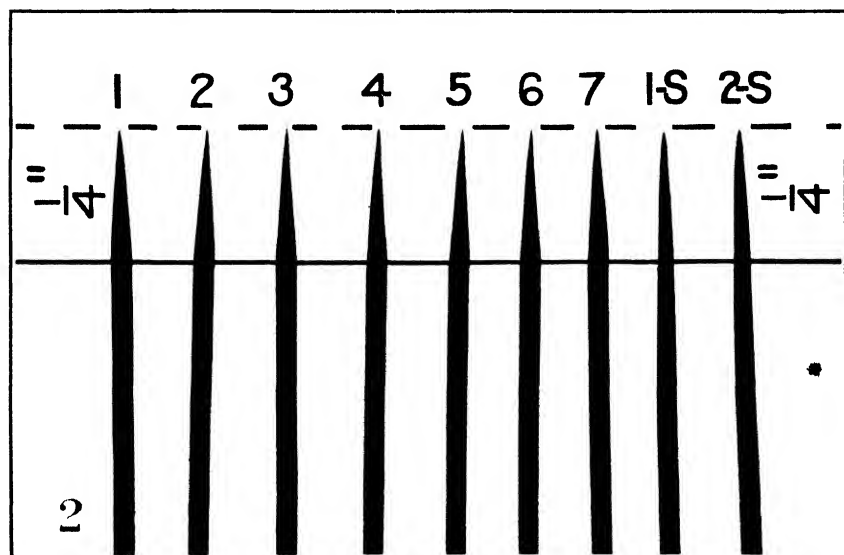
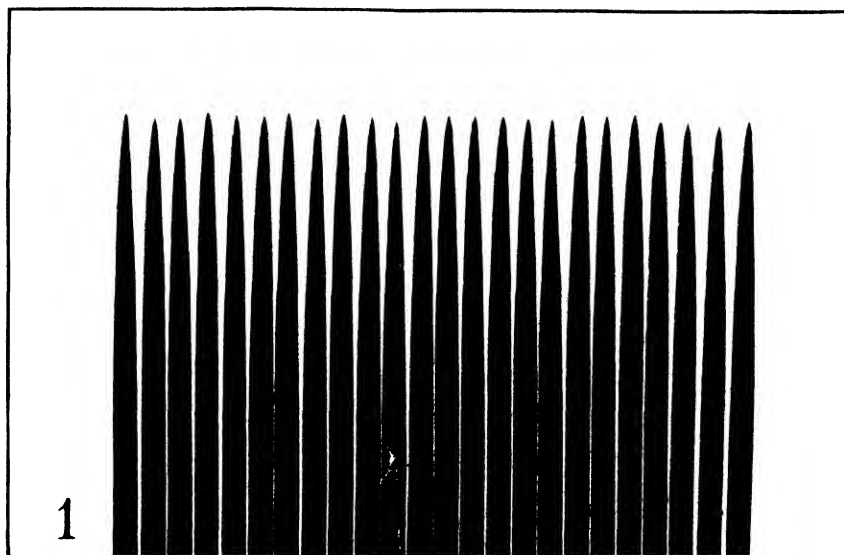
<sup>1</sup> The tempering solution consisted of 5 teacupfuls of common salt, 6 ounces of saltpeter, 12 teaspoonfuls of powdered alum, and 1 teaspoonful of corrosive sublimate dissolved in 10 gallons of water. The needle was tempered by heating carefully to a dull white heat and plunging at once into the tempering solution. It was then lightly cleaned with smooth emery cloth, heated carefully to a point below dull redness, and again plunged into the solution.



### PLATE LXXXII

Fig. 1.—Direct enlargement of a package of No. 2 sewing needles, showing the variations in shape.

Fig. 2.—Direct enlargement of penetration needles, showing the comparison between two standard needles (1-S, 2-S) and seven needles of the new type prepared by the writers.





# A NEW IRRIGATION WEIR<sup>1</sup>

By V. M. CONE,

*Irrigation Engineer, Office of Public Roads and Rural Engineering*

## INTRODUCTION

The accurate measurement of water delivered to the irrigator has been retarded by lack of information concerning devices adapted to the various conditions of size and grade of canals, and to the sand and silt troubles encountered throughout the West. These conditions are so varied that it is very improbable that any one type of measuring device will be desirable or practicable for all cases. Although the weir is the principal measuring device in use in the West, there are many places where the common types of weirs can not be used, and consequently water users are either making current-meter measurements occasionally or systematically or are doing without any measurement.

Many attempts have been made to devise a weir that would be simple and inexpensive in construction, free from sand troubles, and accurate and simple in operation; but usually what has been gained in one direction has been lost in another.

Weirs with full contractions have been built in many places where sand and silt accumulations have resulted in inaccurate measurements, or constant attention has been required to keep the weir box clean. The first cost of such a weir is rather high and the nuisance and expense of keeping it clean often make it undesirable. In an attempt to overcome these objections many weirs have been built with incomplete contractions which have caused the water to pass through the weir box at a velocity sufficiently high to necessitate the addition of a correction factor to the discharge table, but not high enough to completely prevent the accumulation of sand. It usually occurs that full-contraction-weir tables without correction are used with the modified weirs, and therefore the measurement is not worth much more than the guess of an experienced ditch rider. Damage has resulted from the prevalent belief that the weirs in general carry the stamp of accuracy. Under proper conditions of construction and operation, full-contracted weirs are accurate within a small percentage,<sup>2</sup> but such conditions are not always to be found in the field. In the literature of hydraulics there are practically no records of

<sup>1</sup> The work on which this paper is based was done in the hydraulic laboratory, at Fort Collins, Colo., under a cooperative agreement between the Office of Experiment Stations, United States Department of Agriculture, and the Colorado Agricultural Experiment Station.

<sup>2</sup> Cone, V. M. Flow through weir notches with thin edges and full contractions. *In Jour. Agr. Research*, v. 5, no. 23, p. 1051-1114, 1916.

experiments with weirs having completely suppressed bottom contraction. The idea previously considered seems to have been the suppression of the end contractions in order to secure a simple discharge formula, but such an arrangement of weir box possesses many of the objectionable features of full-contracted weirs. Discharge formulas are infrequently used in the field, tables usually being available, and it therefore seems preferable to have a weir that is practicable and of permanent accuracy rather than to complicate the weir-box conditions in order to simplify

the discharge formula. A series of experiments was made in the hydraulic laboratory at Fort Collins, Colo., during the summer of 1914, for the purpose of developing a weir that would be self-cleaning, require a minimum amount of labor and material for construction, measure discharges with an accuracy commensurate with field conditions and irrigation demands, and be easily operated by the ordinary

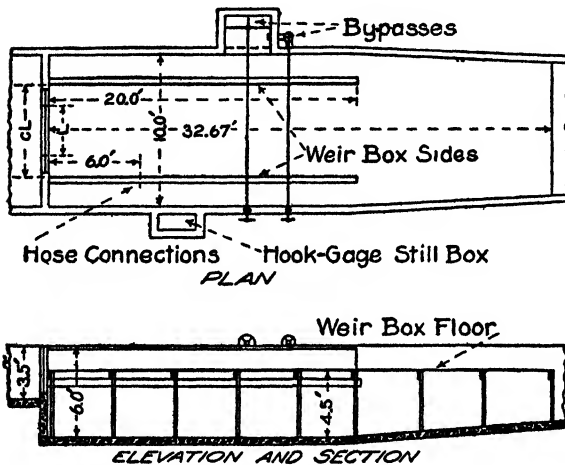


FIG. 1.—Plan, elevation, and section of concrete weir box in the hydraulic laboratory of the Colorado Experiment Station; also arrangement of experimental weir section for Nos. 1 to 6 and 13 to 16, in Table I.

nary man, which means that only simple readings without any computations would be required to determine the discharge.

#### ARRANGEMENT OF APPARATUS FOR EXPERIMENTS WITH NEW TYPE OF WEIR <sup>1</sup>

In the permanent concrete weir box, which is 10 feet wide and 6 feet deep, a wood floor was built of tongue-and-groove lumber (fig. 1). The wood floor was about 4.5 feet above the concrete floor and was water-tight and level throughout. Its length was 20 feet for four sets of experiments, but it was extended to 32.67 feet for all other experiments. The sides of the temporary weir box were made of single widths of boards set in a vertical position, but arranged to be moved to any position or any angle and rigidly fastened to the floor. The several arrangements of the weir box are given in Table I, and figures 1 to 13, inclusive.

<sup>1</sup> For a description of the hydraulic laboratory and equipment, see Cone, V. M., op. cit., and Cone, V. M., Hydraulic laboratory for irrigation investigations, Fort Collins, Colo. *In* Engin. News, v. 70, no. 14, p. 662-665, 5 fig., 1913.



TABLE I.—Effect of size and shape of weir box on discharge<sup>1</sup>

## SPECIAL TESTS

No.	Length of weir crest.	Width of weir box at crest.	Width of weir box at 20 feet.	Equation of discharge curve.	Fig. No.	Length of floor.	Remarks.
	<i>Feet.</i>					<i>Feet.</i>	
1	1	1½ L	1½ L	$Q=4.641 LH^{1.675}$	1	32.67	Sides parallel, no wings.
2	1	2 L	2 L	$Q=3.768 LH^{1.622}$	1	32.67	Do.
3	1	3 L	3 L	$Q=3.441 LH^{1.498}$	1	32.67	Do.
4	1	4 L	4 L	$Q=3.343 LH^{1.489}$	1	32.67	Do.
5	1	5 L	5 L	$Q=3.316 LH^{1.484}$	1	32.67	Do.
6	1	6 L	6 L	$Q=3.274 LH^{1.479}$	1	32.67	Do.
7	1	2 L	3 L	$Q=3.72 LH^{1.627}$	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
8	1	2 L	3 L	$Q=3.69 LH^{1.606}$	3	32.67	Sides extended to sides of concrete box at angle of 45° to axis.
9	1	2 L	3 L	$Q=3.71 LH^{1.622}$	4	32.67	Sides extended to sides of concrete box at angle of 90° to axis.
10	1	2 L	2½ L	$Q=3.73 LH^{1.618}$	4	32.67	Do.
11	1	2 L	2½ L	$Q=3.73 LH^{1.610}$	3	32.67	Sides extended to sides of concrete box at angle of 45° to axis.
12	1.5	2 L	3 L	$Q=3.64 LH^{1.623}$	2	...	Sides extended at same angle to distance of 32.5 feet from crest.
13	2	1½ L	1½ L	$Q=4.375 LH^{1.688}$	1	32.67	Sides parallel, no wings.
14	2	2 L	2 L	$Q=3.749 LH^{1.603}$	1	32.67	Do.
15	2	2½ L	2½ L	$Q=3.552 LH^{1.538}$	1	32.67	Do.
16	2	3 L	3 L	$Q=3.439 LH^{1.685}$	1	32.67	Do.
17	2	2 L	2 L	$Q=3.749 LH^{1.646}$	5	32.67	Sides parallel, with 45° wings connecting parallel sides 12 feet long, 3 L apart.
18	2	2 L	3 L	$Q=3.63 LH^{1.640}$	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
19	3	2 L	2½ L	$Q=3.640 LH^{1.600}$	6	32.67	Sides extended 12 feet parallel to axis and 2½ L apart.
20	3	2 L	3 L	$Q=3.604 LH^{1.600}$	2	32.67	Sides extended about 5 feet at same angle to sides of concrete box.
21	4	1½ L	1½ L	$Q=5.327 LH^{1.698}$	7	20.00	Sides parallel, no wings.
22	4	1½ L	1½ L	$Q=4.105 LH^{1.599}$	7	20.00	Do.
23	4	1½ L	1½ L	$Q=4.053 LH^{1.604}$	2	32.67	Sides parallel, extended to distance of 32.5 feet from crest.
24	4	1½ L	1½ L	$Q=3.839 LH^{1.688}$	7	20.00	Sides parallel, no wings.
25	4	2 L	2 L	$Q=3.599 LH^{1.607}$	7	20.00	Do.
26	4	2 L	2 L	$Q=3.590 LH^{1.600}$	2	32.67	Sides parallel, extended to distance of 32.5 feet from crest.
27	4	2 L	2 L	$Q=3.714 LH^{1.670}$	8	32.67	Sides parallel, with 45° wings extending to sides of concrete box.
28	4	2 L	2 L	$Q=3.642 LH^{1.642}$	9	32.67	Sides parallel, with 90° wings extending to sides of concrete box.
29	4	2½ L	2½ L	$Q=3.403 LH^{1.600}$	10	32.67	Full width of concrete box.

## STANDARD TESTS

30	1	2 L	2½ L	$Q=3.771 LH^{1.63}$	2	32.67	Sides extended at same angle to distance of 32.5 feet from crest.
31	1.5	2 L	2½ L	$Q=3.720 LH^{1.64}$	2	32.67	Do.
32	2	2 L	2½ L	$Q=3.690 LH^{1.64}$	2	32.67	Do.
33	3	2 L	2½ L	$Q=3.630 LH^{1.65}$	2	32.67	Do.
34	4	2 L	2½ L	$Q=3.570 LH^{1.66}$	2	32.67	Sides extended at same angle to sides of concrete box.

## SPECIAL NOTCH TESTS

35	Degrees. 90	Feet. 10	Feet. 10	$Q=2.541 H^{2.62}$	11	32.67	No sides, channel full width of concrete box.
36	90	3	7	$Q=2.667 H^{2.81}$	12	32.67	Sides extended about 10 feet at same angle to sides of concrete box.
37	90	3	.....	$Q=2.679 H^{2.817}$	13	32.67	Sides 5 feet apart at 10 feet, then extended 12 feet parallel to axis.

<sup>1</sup> Level wood floor placed about 4.5 feet above floor of concrete weir box; angle iron weir crest.

Steel weir plates having rectangular crests and sides made of brass, with nominal crest lengths of 1, 1.5, 2, 3, and 4 feet, were successively attached to the steel frame anchored in the concrete wall. A 2-inch angle iron, dressed and trued, was set flush in the floor section, and by means of bolts the floor section was drawn tightly against the weir plate. The angle iron formed the crest of the weir and it was sufficiently rigid to prevent any trouble due to the possible warping of the floor, and also insured the crests remaining at the same elevation as the floor. The water passed through the weir notch with full lateral expansion and complete aeration of nappe.

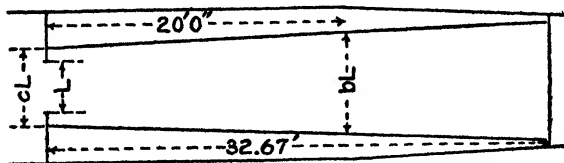


FIG. 2.—Plan of experimental weir box for Nos. 7, 12, 18, 20, and 30 to 34 in Table I.

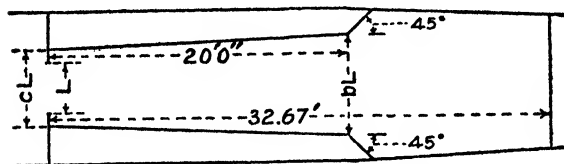


FIG. 3.—Plan of experimental weir box for Nos. 8 and 11, Table I.

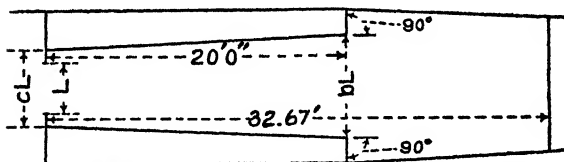


FIG. 4.—Plan of experimental weir box for Nos. 9 and 10, Table I.

temporary still box connected by a hose through the side of the weir box near the floor line. This hook gauge was used for check purposes and to determine whether any discrepancies would be introduced by applying the results of the experiments to future installations where the head would be communicated to a still box by pipes through the side of the weir box. The two sets of hook-gauge readings indicated that no error is introduced thereby, provided the pipes are installed at the proper distance from the weir, 6 feet, and in a position normal to the side of the weir box rather than normal to the axis, because the lines of flow are parallel to the side.

In all these experiments the weir discharges were determined volumetrically in the calibrated concrete tanks.

Several series of preliminary experiments were made in order to determine the influence upon the discharge caused by various end contrac-

The head was determined in the concrete hook-gauge still box which was connected to the weir box by four pieces of  $\frac{3}{4}$ -inch hose attached to 1-inch pipe nipples screwed upward through the floor until flush with the surface. The auger holes into which the pipes were screwed were placed near the side of the weir box in a line 6 feet back from the plane of the weir. A second hook gauge was placed in a tem-

tion distances, lengths of weir box, contraction wings at entrance of weir box, and angle of sides of weir box. From these data a set of conditions was chosen to be the standard for the new type of weir, for it is obviously necessary that the weir box be definitely standardized in order that the specifications be duplicated in future installations if the formula and tables are to apply. The terms "standard tests" or "standard conditions" will be used to express those conditions which have been taken as the basis of the formula and discharge tables.

The water passes through the weir box with a rather high velocity, but the velocity varies with the head, and the slope of the water surface changes accordingly. The extent of the draw-down curve also varies with the head and length of weir crest and it was therefore necessary to fix the point at which to take the head. Several measurements of draw-down curves resulted in choosing a point 6 feet back from the plane of the weir, which would be away from any considerable influence of draw-down for the weirs used in the experiments, and would not include much of the slope of the water surface.

A total of 277 experiments were made on this new type of weir, which for want of a better name is called an "irrigation weir," and of this number 101 were preliminary tests and 176 were made under standard conditions.

#### DEDUCTIONS FROM EXPERIMENTS

The individual equations in simple form for each set of experiments and the conditions under which those experiments were made are given in Table I. The following deductions have been obtained from comparisons of the equations stated in the table, the bottom contractions being entirely suppressed in all cases, but with various arrangements of sides of weir box.

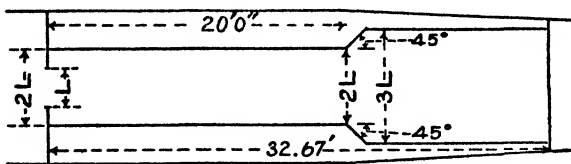


FIG. 5.—Plan of experimental weir box for No. 17, Table I.

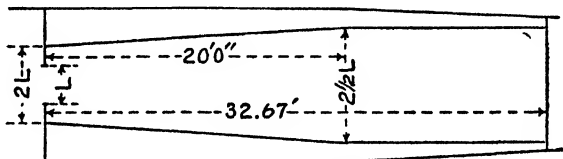


FIG. 6.—Plan of experimental weir box for No. 19, Table I.

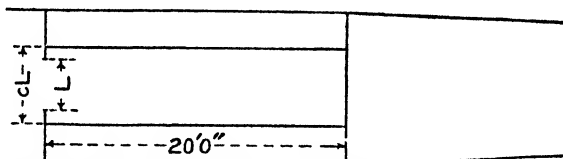


FIG. 7.—Plan of experimental weir box for Nos. 21, 22, 24, and 25, Table I.

For similar conditions of weir box, the coefficient  $c$  decreases as the length of weir crest  $L$  increases, and the exponent  $n$  increases as the length increases.

As the width of weir box, or end contractions, is increased for any certain length of weir, both  $c$  and  $n$  decrease. This is probably due to a decrease in the velocity of approach, owing to the increased area of the weir box.

When the sides of the weir box are parallel, the discharge increases as the width of the box is decreased, for all sizes of weirs.

The greatest discharge is obtained when the sides of the weir box are parallel and it decreases as the angle between the sides becomes greater;

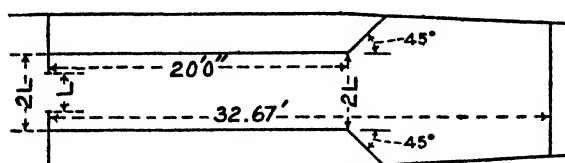


FIG. 8.—Plan of experimental weir box for No. 27, Table I.

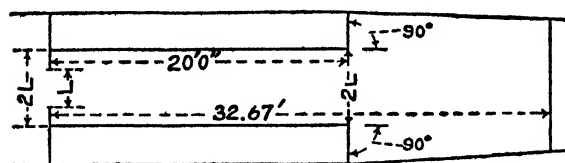


FIG. 9.—Plan of experimental weir box for No. 28, Table I.

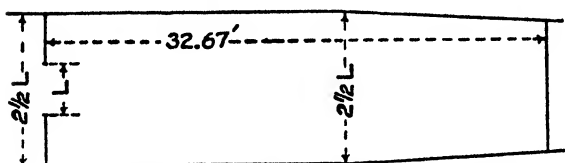


FIG. 10.—Plan of experimental weir box for No. 29, Table I.

or, stated in another way, the discharge increases as the sides become more nearly parallel, the width of the box at the weir remaining constant.

When wings placed at the upper end of the weir box to form a junction between the sides of the box and the canal bank are changed from  $90^\circ$  to  $45^\circ$  with the axis of the channel, the discharge is increased for low heads, remains about the same for heads of 0.7 foot, and is decreased for high

heads. The percentage of change in discharge due to such a change in the wings is greater when the sides of the weir box are parallel.

The ratio of discharge to length of weir decreases as the length of the weir increases; or, in other words, the discharge over a 4-foot weir is less than four times the discharge over a 1-foot weir, as is shown by the individual standard equations, Nos. 30 to 34, in Table I. This is the reverse of the condition found in rectangular weirs having complete end and bottom contractions and negligible velocity of approach.<sup>1</sup>

If the sides of the weir box are continued parallel from a point 20 feet upstream from the plane of the weir (fig. 6), instead of being continued

<sup>1</sup> Cone, V. M. Flow through weir notches with thin edges and full contractions. *In Jour. Agr. Research*, v. 5, no. 23, p. 1051-1114. 1916.

at the same angle as the other part of the weir box (fig. 2), the discharge will be increased about one-third of 1 per cent for 1-foot head and decreased about 1 per cent for 0.2-foot head, as indicated for the 3-foot weir in Nos. 19 and 33 in Table I.

In addition to the experiments with regular weir notches, three sets of experiments were made with 90° triangular notches having suppressed bottom contraction and different end contractions. The results are represented by Nos. 35, 36, and 37 in Table I. The logarithmic discharge curve for the 90° triangular notch with complete end and bottom contractions is a perfect straight line represented by the equation  $Q = 2.487h^{2.4806}$ . Suppression of the bottom contraction, No. 35 in Table I, resulted in changing the logarithmic discharge curve from a straight line to a curved line, and increased the discharge. An average straight line drawn through the discharge data, represented by the equation  $Q = 2.541h^{2.492}$ , agrees with the experimental data for medium heads, but is about 1 per cent low for high and low heads.

The second set of experiments, No. 36 in Table I, also gave a logarithmic plot which was a curved line.

The average straight line for these data was about 1 per cent low for heads of 0.3 and 1.3 feet, and about 2 per cent high for heads of approximately 0.8 foot. This indicates the curvature of the discharge plot to be increased by a decrease in end-contraction distances.

The third set of experiments, No. 37 in Table I, was made under conditions which practically amounted to making the weir box 10 feet shorter than in the previous case, having the sides of the carrying channel parallel in both cases, but closer together in this set of experiments. This had little effect upon the discharge in the aggregate, but changed the slope of the discharge curve slightly.

The 90° triangular notch with full contractions is one of the most accurate and reliable measuring devices for small quantities of water.

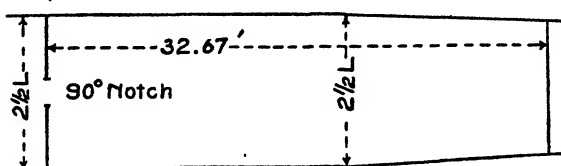


FIG. 11.—Plan of experimental weir box for No. 35, Table I.

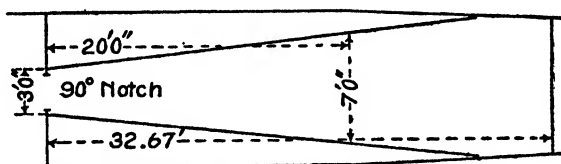


FIG. 12.—Plan of experimental weir box for No. 36, Table I.

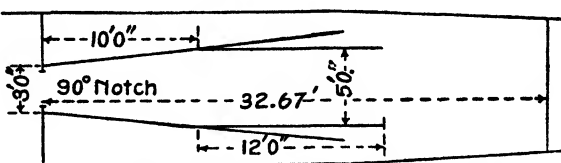


FIG. 13.—Plan of experimental weir box for No. 37, Table I.

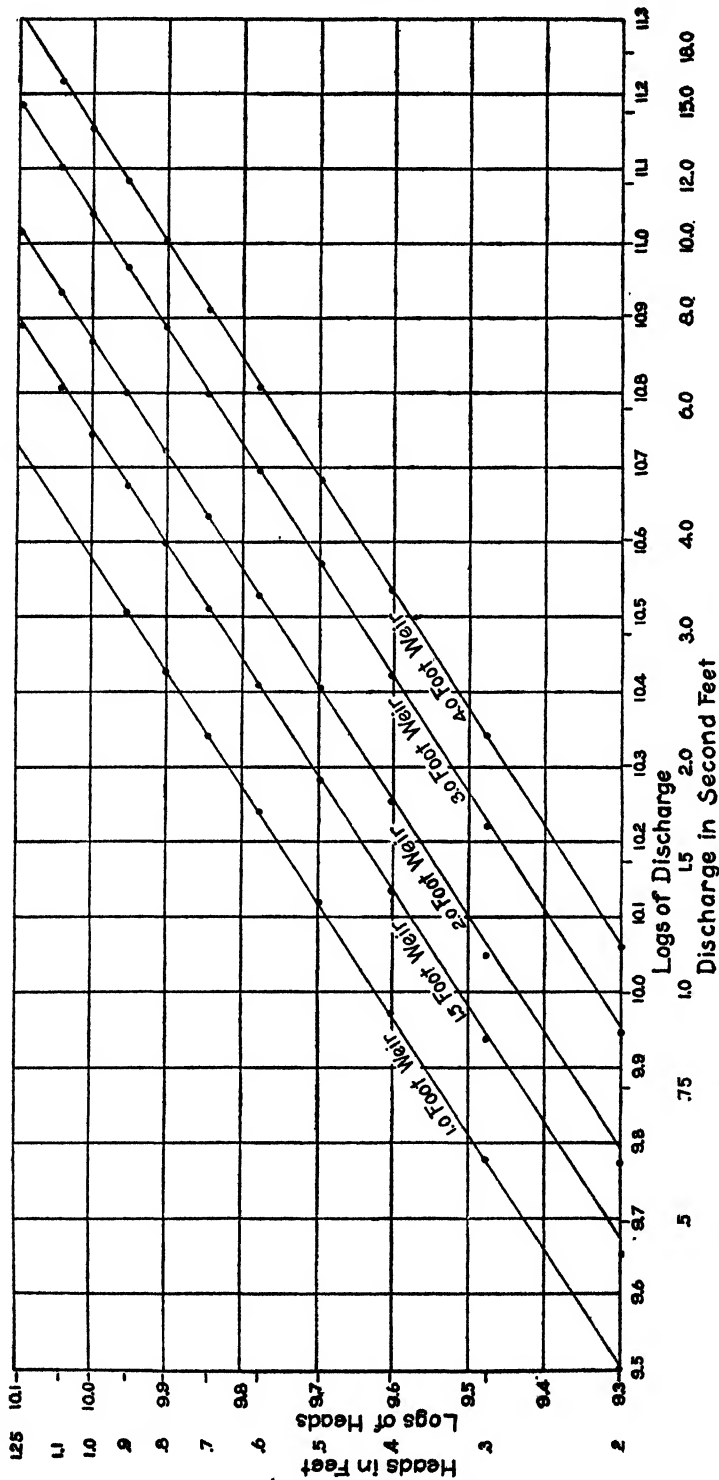


FIG. 14.—Experimental discharge data plotted logarithmically and curves drawn from values computed from standard equation for new irrigation weir.

Suppressing the contractions completely or in part changes the law of discharge through the triangular notch, decreases its accuracy as a practical measuring device, and does not insure the complete removal of sand and silt from the box. It is therefore an open question whether the advantages resulting from suppressed contractions with the triangular notch would not be more than counterbalanced by the inaccuracies introduced. The data are given without recommendation, but may be desirable for use in special cases.

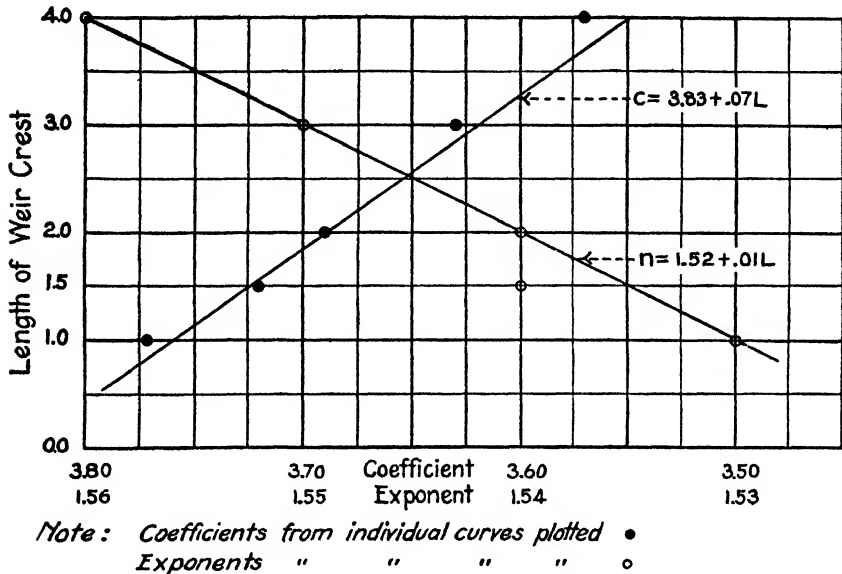


FIG. 15.—Coefficient and exponent values of individual discharge equations plotted against weir length.

#### DERIVATION OF WEIR FORMULA

The experimental discharge data for the standard weir conditions were plotted logarithmically for weirs having actual crest lengths of 1.0055, 1.5026, 2.0057, 2.9970, and 4.0056 feet, as shown in figure 14. These points do not lie on a straight line. An average straight line drawn through the points will give values too small for medium heads and too large for low and high heads. This characteristic of the curve is the reverse of the curve for rectangular weirs with full contractions, but the suppression of the bottom contraction and partial suppression of the end contraction has tended to straighten the discharge curve.

With full-contraction weirs and quite complete pondage, the head can be accurately determined and there is, therefore, ample reason for using a complicated formula to secure that accuracy of measurement, but the high velocity of water and wave action which occurs in the new irrigation weir preclude the possibility of determining the head accu-

ately enough to warrant any great refinement of the discharge formula. The assumption of straight-line logarithmic formulas is within 1 or 2 per cent of all the discharge data, with the exception of a few high and low heads; and since this is comparable to the accuracy expected under field conditions, such formulas were used to avoid more complicated equations.

The equations of the average straight lines through the plotted points are given in Table I, Nos. 30 to 34, inclusive. The exponent and coefficient values for these individual equations were then plotted against the weir crest lengths, as shown in figure 15. For simplicity the law of the

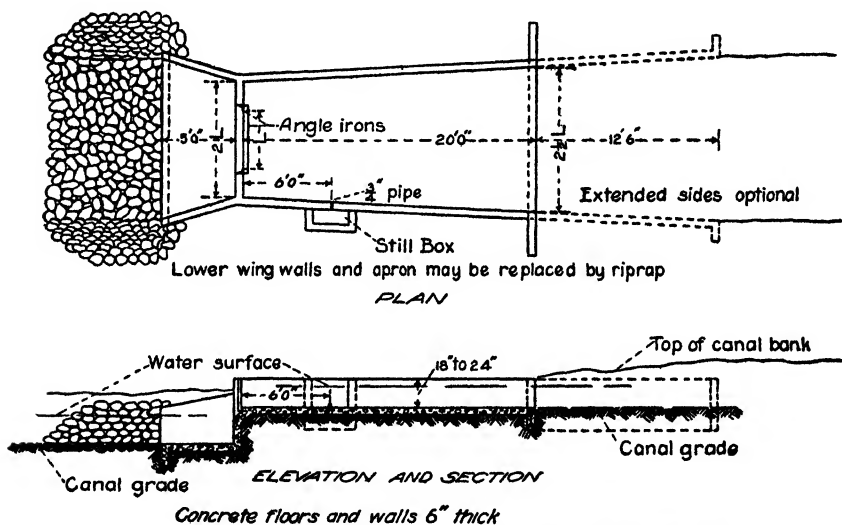


FIG. 16.—Plan, elevation, and section (standard) of new irrigation weir box.

coefficient values was assumed to be represented by the equation  $c = (3.83 - 0.07L)$ . The exponents, with the single exception of that for the 1.5-foot weir, fell on the straight line which has the equation  $n = (1.52 + 0.01L)$ . By substituting these expressions in the fundamental formula,  $Q = cLh^n$ , the general formula for the new irrigation weir was obtained

$$Q = (3.83 - 0.07L)Lh^{(1.52 + 0.01L)}$$

The straight-line curves drawn in figure 14 for each length of weir represent discharge values computed from the above formula and show graphically the agreement of the formula with the experimental data. The computed discharges are given in Table II.



TABLE II.—Computed discharges for the new irrigation weirs

[Computed from the formula  $Q = (3.83 - 0.07 L) Lh^{(1.02+0.01 L)}$ ]

Head.	Head.	Length of weir crest.				
		1 foot.	1 5 feet.	2 feet.	3 feet.	4 feet.
<i>Feet.</i>	<i>Feet. in.</i>					
0.20	2 3/8	0.320	0.472	0.619	0.896	1.15
.21	2 1/4	.345	.509	.667	.966	1.24
.22	2 3/8	.371	.547	.717	1.04	1.34
.23	2 3/4	.397	.586	.768	1.11	1.43
.24	2 7/8	.424	.625	.820	1.19	1.53
.25	3	.451	.665	.873	1.27	1.63
.26	3 1/8	.479	.707	.927	1.34	1.74
.27	3 1/4	.507	.749	.982	1.43	1.84
.28	3 3/8	.536	.792	1.04	1.51	1.95
.29	3 1/2	.566	.836	1.10	1.59	2.06
.30	3 5/8	.596	.880	1.16	1.68	2.17
.31	3 3/4	.626	.926	1.22	1.77	2.28
.32	3 7/8	.658	.972	1.28	1.86	2.40
.33	3 1/2	.690	1.02	1.34	1.95	2.52
.34	4 1/8	.722	1.07	1.40	2.04	2.64
.35	4 1/4	.754	1.12	1.47	2.13	2.76
.36	4 3/8	.788	1.16	1.53	2.23	2.88
.37	4 1/2	.822	1.21	1.60	2.33	3.01
.38	4 5/8	.856	1.27	1.66	2.42	3.14
.39	4 3/4	.890	1.32	1.73	2.52	3.27
.40	4 7/8	.925	1.37	1.80	2.62	3.40
.41	4 1/2	.961	1.42	1.87	2.73	3.53
.42	5 1/8	.997	1.48	1.94	2.83	3.67
.43	5 1/4	1.03	1.53	2.01	2.94	3.81
.44	5 3/8	1.07	1.58	2.08	3.04	3.94
.45	5 1/2	1.11	1.64	2.16	3.15	4.09
.46	5 5/8	1.15	1.70	2.23	3.26	4.23
.47	5 3/4	1.18	1.75	2.31	3.37	4.37
.48	5 7/8	1.22	1.81	2.38	3.48	4.52
.49	5 1/2	1.26	1.87	2.46	3.59	4.67
.50	6	1.30	1.93	2.54	3.71	4.82
.51	6 1/8	1.34	1.99	2.62	3.82	4.97
.52	6 1/4	1.38	2.05	2.70	3.94	5.12
.53	6 3/8	1.42	2.11	2.78	4.06	5.27
.54	6 1/2	1.46	2.17	2.86	4.18	5.43
.55	6 5/8	1.51	2.23	2.94	4.30	5.59
.56	6 3/4	1.55	2.29	3.02	4.42	5.75
.57	6 7/8	1.59	2.36	3.11	4.54	5.91
.58	6 1/2	1.63	2.42	3.19	4.67	6.07
.59	7 1/8	1.68	2.49	3.27	4.79	6.23
.60	7 1/4	1.72	2.55	3.36	4.92	6.40
.61	7 3/8	1.76	2.62	3.45	5.05	6.57
.62	7 1/2	1.81	2.68	3.53	5.18	6.74
.63	7 5/8	1.85	2.75	3.62	5.31	6.91
.64	7 1/2	1.90	2.82	3.71	5.44	7.08
.65	7 7/8	1.95	2.88	3.80	5.57	7.25
.66	7 3/4	1.99	2.95	3.89	5.70	7.43
.67	8 1/8	2.04	3.02	3.98	5.84	7.60
.68	8 1/4	2.08	3.09	4.08	5.97	7.78
.69	8 3/8	2.13	3.16	4.17	6.11	7.96

TABLE II.—Computed discharges for the new irrigation weirs—Continued

Head.	Head.	Length of weir crest.				
		1 foot.	1.5 feet.	2 feet.	3 feet.	4 feet.
<i>Feet.</i>	<i>Feet.</i>					
0.70	0 8 $\frac{3}{8}$	2.18	3.23	4.26	6.25	8.14
.71	0 8 $\frac{1}{2}$	2.23	3.30	4.35	6.39	8.32
.72	0 8 $\frac{5}{8}$	2.27	3.37	4.45	6.53	8.50
.73	0 8 $\frac{3}{4}$	2.32	3.45	4.55	6.67	8.69
.74	0 8 $\frac{7}{8}$	2.37	3.52	4.64	6.81	8.88
.75	0 9	2.42	3.59	4.74	6.95	9.06
.76	0 9 $\frac{1}{8}$	2.47	3.67	4.84	7.10	9.25
.77	0 9 $\frac{1}{4}$	2.52	3.74	4.94	7.24	9.44
.78	0 9 $\frac{3}{8}$	2.57	3.82	5.03	7.39	9.64
.79	0 9 $\frac{1}{2}$	2.62	3.89	5.13	7.54	9.83
.80	0 9 $\frac{5}{8}$	2.67	3.97	5.23	7.68	10.02
.81	0 9 $\frac{3}{4}$	2.72	4.04	5.34	7.83	10.22
.82	0 9 $\frac{7}{8}$	2.78	4.12	5.44	7.98	10.42
.83	0 9 $\frac{1}{2}$	2.83	4.20	5.54	8.14	10.62
.84	0 10 $\frac{1}{8}$	2.88	4.28	5.64	8.29	10.82
.85	0 10 $\frac{3}{8}$	2.93	4.35	5.75	8.44	11.02
.86	0 10 $\frac{1}{4}$	2.99	4.43	5.85	8.60	11.22
.87	0 10 $\frac{5}{8}$	3.04	4.51	5.95	8.75	11.43
.88	0 10 $\frac{3}{4}$	3.09	4.59	6.06	8.91	11.63
.89	0 10 $\frac{7}{8}$	3.15	4.67	6.17	9.07	11.84
.90	0 10 $\frac{1}{2}$	3.20	4.75	6.27	9.22	12.05
.91	0 10 $\frac{5}{8}$	3.25	4.83	6.38	9.38	12.26
.92	0 11 $\frac{1}{8}$	3.31	4.92	6.49	9.54	12.47
.93	0 11 $\frac{3}{8}$	3.36	5.00	6.60	9.70	12.68
.94	0 11 $\frac{1}{4}$	3.42	5.08	6.71	9.87	12.89
.95	0 11 $\frac{5}{8}$	3.48	5.16	6.82	10.03	13.11
.96	0 11 $\frac{3}{4}$	3.53	5.25	6.93	10.19	13.32
.97	0 11 $\frac{7}{8}$	3.59	5.33	7.04	10.36	13.54
.98	0 11 $\frac{1}{2}$	3.65	5.42	7.15	10.53	13.76
.99	0 11 $\frac{5}{8}$	3.70	5.50	7.27	10.69	13.98
1.00	1 0	3.76	5.59	7.38	10.86	14.20
1.01	1 0 $\frac{1}{8}$	3.82	5.67	7.49	11.03	14.42
1.02	1 0 $\frac{1}{4}$	3.88	5.76	7.61	11.20	14.64
1.03	1 0 $\frac{3}{8}$	3.93	5.85	7.72	11.37	14.87
1.04	1 0 $\frac{1}{2}$	3.99	5.93	7.84	11.54	15.10
1.05	1 0 $\frac{5}{8}$	4.05	6.02	7.96	11.71	15.32
1.06	1 0 $\frac{3}{4}$	4.11	6.11	8.07	11.89	15.55
1.07	1 0 $\frac{7}{8}$	4.17	6.20	8.19	12.06	15.78
1.08	1 0 $\frac{1}{2}$	4.23	6.29	8.31	12.24	16.01
1.09	1 1 $\frac{1}{8}$	4.29	6.38	8.43	12.41	16.24
1.10	1 1 $\frac{3}{8}$	4.35	6.47	8.55	12.59	16.48
1.11	1 1 $\frac{1}{4}$	4.41	6.56	8.66	12.77	16.71
1.12	1 1 $\frac{5}{8}$	4.47	6.65	8.79	12.94	16.95
1.13	1 1 $\frac{3}{4}$	4.53	6.74	8.91	13.12	17.18
1.14	1 1 $\frac{7}{8}$	4.59	6.83	9.03	13.30	17.42
1.15	1 1 $\frac{1}{2}$	4.66	6.92	9.15	13.49	17.66
1.16	1 1 $\frac{5}{8}$	4.72	7.02	9.28	13.67	17.90
1.17	1 1 $\frac{3}{4}$	4.78	7.11	9.40	13.85	18.14
1.18	1 1 $\frac{7}{8}$	4.84	7.20	9.52	14.04	18.38
1.19	1 2 $\frac{1}{4}$	4.91	7.30	9.65	14.22	18.63

TABLE II.—Computed discharges for the new irrigation weirs—Continued

Head.	Head.	Length of weir crest.				
		1 foot.	1.5 feet.	2 feet.	3 feet.	4 feet.
<i>Feet.</i>	<i>Ft. in.</i>					
1. 20	1 2 $\frac{3}{8}$	4. 97	7. 39	9. 77	14. 41	18. 87
1. 21	1 2 $\frac{1}{2}$	5. 03	7. 49	9. 90	14. 59	19. 12
1. 22	1 2 $\frac{5}{8}$	5. 10	7. 58	10. 02	14. 78	19. 36
1. 23	1 2 $\frac{3}{4}$	5. 16	7. 68	10. 15	14. 97	19. 61
1. 24	1 2 $\frac{7}{8}$	5. 23	7. 77	10. 28	15. 16	19. 86
1. 25	1 3	5. 29	7. 87	10. 41	15. 35	20. 11

Table III shows the differences between the discharges computed from the formula and those obtained by experiment, these differences being expressed in cubic feet per second and in percentages. The formula agrees with the experimental data within a maximum amount of 4.8 per cent for an individual point, but this discrepancy is no doubt due partly to experimental inaccuracy and partly to the assumption of a straight-line formula. Medium heads give values for discharges that agree within 1 per cent, but the high and low heads will have a somewhat greater error. The formula agrees with the average straight lines drawn through the experimental data within a maximum error of 1 per cent. The error is greatest with the small weirs, decreases as the length of the weir increases, and for a length of 4 feet the error is quite small. Although the formula is derived from experiments with weirs having a maximum length of 4 feet it seems probable that the formula will be even closer for weirs with greater crest lengths.

TABLE III.—Difference between discharges computed from the formula  $Q = [3.83 - 0.07L]LH^{1.52+0.01L}$  and those obtained by experiment, for the new type of weir

## 1-FOOT WEIR

Head.	Observed $Q$ corrected true for length.	Computed $Q$ .	Difference in $Q$ .	Percentage of difference. <sup>1</sup>
<i>Feet.</i>				
0. 200.....	0. 314	0. 320	+0. 006	+1. 94
. 300.....	. 595	. 596	+ . 001	+ . 17
. 400.....	. 935	. 925	+ . 010	+1. 07
. 500.....	1. 299	1. 302	+ . 003	+ . 20
. 599.....	1. 727	1. 716	- . 011	- . 60
. 699.....	2. 183	2. 174	- . 009	- . 40
. 800.....	2. 661	2. 673	+ . 012	+ . 50
. 895.....	3. 113	3. 173	+ . 060	+1. 92

<sup>1</sup> Percentage of difference between discharge obtained by computations from the formula  $Q = [3.83 - 0.07L]LH^{1.52+0.01L}$  and by experiment, the bases of comparison being the experimental data.

TABLE III.—Difference between discharges computed from the formula  $Q=[3.83-0.07L]LH^{1.82+0.01L}$  and those obtained by experiment, for the new type of weir—Continued

## 1.5-FOOT WEIR

Head.	Observed $Q$ corrected true for length.	Computed $Q$ .	Difference in $Q$ .	Percentage of difference.
<i>Feet.</i>				
0.199.....	0.448	0.460	+0.021	+4.69
.299.....	.865	.876	+ .011	+1.30
.400.....	1.360	1.369	+ .009	+ .66
.497.....	1.907	1.910	+ .003	+ .16
.600.....	2.560	2.551	- .009	- .35
.700.....	3.227	3.232	+ .005	+ .15
.800.....	3.956	3.967	+ .011	+ .28
.900.....	4.728	4.753	+ .025	+ .53
.998.....	5.521	5.570	+ .049	+ .89
1.099.....	6.378	6.459	+ .081	+1.27
1.250.....	7.727	7.870	+ .143	+1.85

## 2-FOOT WEIR

0.200.....	0.590	0.619	+0.029	+4.80
.300.....	1.116	1.156	+ .040	+3.58
.400.....	1.784	1.800	+ .016	+ .90
.500.....	2.536	2.538	+ .002	+ .08
.600.....	3.358	3.361	+ .003	+ .09
.700.....	4.288	4.261	- .027	- .63
.800.....	5.179	5.234	+ .055	+1.06
.900.....	6.279	6.274	- .005	- .08
1.000.....	7.358	7.380	+ .022	+ .30
1.100.....	8.540	8.547	+ .007	+ .08
1.250.....	10.335	10.406	+ .071	+ .69

## 3-FOOT WEIR

0.200.....	0.884	0.896	+0.012	+1.36
.300.....	1.663	1.680	+ .017	+1.02
.396.....	2.583	2.584	+ .001	+ .04
.501.....	3.720	3.720	.000	.00
.598.....	4.938	4.895	- .043	- .85
.700.....	6.297	6.248	- .049	- .78
.800.....	7.754	7.684	- .070	- .90
.900.....	9.287	9.223	- .064	- .69
1.001.....	10.948	10.877	- .071	- .65
1.100.....	12.638	12.589	- .049	- .39
1.250.....	15.331	15.347	+ .016	+ .10

TABLE III.—Difference between discharges computed from the formula  $Q=[3.83-0.07L]LH^{(1.82+0.01L)}$  and those obtained by experiment, for the new type of weir—Continued

## 4-FOOT WEIR

Head.	Observed $Q$ corrected true for length.	Computed $Q$ .	Difference in $Q$ .	Percentage of difference.
<i>Feet.</i>				
0.200.....	1.148	1.153	+0.005	+0.44
.301.....	2.188	2.182	— .006	— .27
.399.....	3.417	3.387	— .030	— .88
.500.....	4.806	4.817	+ .011	+ .23
.601.....	6.427	6.417	— .010	— .16
.700.....	8.158	8.141	— .017	— .21
.799.....	10.045	10.006	— .039	— .39
.900.....	12.081	12.047	— .034	— .28
1.000.....	14.194	14.200	+ .006	+ .04
1.100.....	16.426	16.476	+ .050	+ .30

SPECIFICATIONS FOR CONSTRUCTION AND USE OF THE NEW  
IRRIGATION WEIR

A plan and elevation of the standard weir is shown in figure 16. The weir notch is rectangular in form, with sharp crest and sides. The floor of the weir box must be level with the crest, and it is therefore convenient to use an angle iron for the crest, embedding one face of the angle until flush with the surface of the floor, the other face of the angle extending downward. The sides of the weir notch may also be made of angle iron placed in a vertical position, with one end extending below the crest and one face of the angle against the angle-iron crest. The angle can then be attached to the weir bulkhead through holes placed in the other face. This arrangement is durable and inexpensive and will meet the requirement of sharp crest and full lateral expansion for the escaping stream of water. The grade of the canal downstream from the weir must be low enough to give free fall and complete aeration to the nappe.

The floor of the weir box must be level throughout, and there must be no sudden or decided differences in elevation between the floor and the grade of the channel of approach. The weir box must be placed in the center of the ditch, so the axial line of the box corresponds with the axial line of the canal, in order that the water may enter the weir box in straight lines. The width of the weir box must be twice the length of the weir crest ( $2L$ ) at the plane of the weir, and two and a half times the length of the weir crest ( $2\frac{1}{2}L$ ) at a distance of 20 feet upstream from the plane of the weir. The standard tests were made with a weir box 32.5 feet long, except for the 4-foot weir, No. 34, Table I, and the sides were extended at the angle indicated above. However, from Table I, Nos. 7, 8, and 9,

and 10, 11, and 30, it will be seen that for the 1-foot weir at least the discharge through a box 32.5 feet long with sides set to the standard dimensions is within 1 per cent of the discharge obtained by placing 90° wings at the end of a similar box 20 feet long. The use of 45° wings will cause an error of about 2½ per cent. Therefore the weir box for the new irrigation weir should be made with sides spaced 2  $L$  at the plane of the weir and 2½  $L$  at 20 feet upstream from the weir, with the sides continuing at this angle until they meet the banks of the ditch or canal; or the box should be only 20 feet long with wings to connect the sides of the box with the canal banks, and these wings should form an angle of 90° with the axis of the weir box. The 90° wings (fig. 2) give a discharge about 1 per cent greater than with the extended sides (fig. 4) for a head of 0.2 foot and about 1 per cent less for a head of 1 foot.

Extending the sides of the weir box until they are the full size of the canal will give more accurate results, but this accuracy may not be required, and the saving in cost of construction due to the shorter length of the weir box with wings may be more desirable than the 1 per cent of accuracy in measuring the water. Unless the canal bottom is easily eroded or scoured, it would not be necessary to extend the floor of the weir box beyond 20 feet, even if the sides of the box are extended.

The comparatively high velocity of the water flowing through the weir box causes a wave action and generally disturbed condition of the water surface, which makes it quite impossible to determine the head  $h$  in the open weir box. Any stilling device placed in the weir would interfere with the action of the weir, and it is therefore necessary that a still box be placed outside the weir box and connected through the side of the weir box with one or more 1-inch pipes located 6 feet from the plane of the weir. The pipe should be placed near the floor of the weir box to insure its being submerged for low heads, and care must be used to place the pipe normal to the side of the weir box, and not normal to the axis of the box. If the pipe is pointed downstream the velocity of the water in the weir box will cause a suction action which will make the water surface in the still box lower than that in the weir box. If the pipe is pointed upstream, there will be a velocity head added to the actual water level in the weir box, and the water in the still box will be higher than that in the weir box. Although no sand or silt will accumulate in the weir box, regardless of the amount carried by the stream, silt may be deposited in the still box and clog the connection pipe unless it is cleaned regularly. By making a deep still box, space will be provided for such silt accumulation and therefore less frequent cleaning will be required. The still box should have inside dimensions of at least 1 foot by 1½ or 2 feet, with such depth as is necessary. The head in the still box may be determined by means of a scale, a hook gauge, or an automatic registering gauge.

The new irrigation weir may be constructed of lumber, but the design is such that it may be easily constructed of concrete. There would be

no difficult form work required for the concrete, and it would make an inexpensive, durable, and satisfactory measuring device, especially if the angle-iron sides and crest of notch were used in connection with the concrete box.

#### ADVANTAGES OF THE NEW IRRIGATION WEIR

(1) The new irrigation weir is self-cleaning. The increasing velocity of the water from the time it enters the weir box until it passes through the weir notch prevents the deposit of sand and silt. Floating materials are also carried through the weir.

(2) No lowering of the canal grade or building up of the banks is required for the construction of the weir box. The weir box has only one-fourth the depth and a less width than is required for a full-contraction weir. Less excavation and less materials are needed in the construction, and the cost of the weir is therefore greatly decreased.

(3) It may be installed by the farmer without expert assistance and with the tools ordinarily at hand. Its operation does not require special training.

(4) Its accuracy is consistent with practical demands and will remain constant.

(5) It can not be easily tampered with or accidentally injured so as to alter its discharge.

(6) There are no working parts which require attention for proper operation. There is practically no upkeep expense if the weir is well constructed of durable materials.

(7) When the discharge tables are used, no computations are required, because the effect of velocity of approach is incorporated in the tables. The weir discharge is expressed in cubic feet per second, which may be converted into any units desired. An automatic recording gauge used in connection with this weir will give a record of the quantity of water discharged at all times, and the aggregate discharge can be computed from the record if desired.

(8) It is not patented, and the entire cost of the weir is for materials and the labor of construction.

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### INHERITANCE OF FERTILITY IN SWINE<sup>1</sup>

[PRELIMINARY PAPER]

By EDWARD N. WENTWORTH, *Professor of Animal Breeding*, and C. E. AUBEL, *Fellow in Animal Breeding, Kansas Agricultural Experiment Station*

#### INTRODUCTION

Mendelian inheritance applies almost without exception to the transmission of qualitative characters. Quantitative traits, on the other hand, are susceptible only to a generalized treatment from this viewpoint, and few investigators have attacked the problem. Size inheritance in animals has been dealt with by Castle and Phillips (2)<sup>2</sup>, Goldschmidt (7), MacDowell (10), Phillips (19, 20), and Punnett and Bailey (21), while Detlefsen (3) has treated the inheritance of certain skeletal characters. Pearl, (15) discovered an arbitrary division point of 30 eggs in the winter laying period of hens, for which inheritance apparently depends on two factors, one of which follows an ordinary Mendelian, and the other a sex-linked scheme. These determiners provide the nearest to units of inheritance that have yet been isolated in quantitative studies.

Because of the fact that fecundity deviates only by discrete units, the litter size in swine provides peculiarly favorable material for studying quantitative inheritance. An analysis of this material has already been attempted from the biometric viewpoint. Rommel and Phillips (24) correlated the size of litters in which dams and daughters were farrowed and found a correlation coefficient of  $0.0601 \pm 0.0086$ . They conclude from this result that there is an actual positive correlation between the size of litters of two successive generations, believing that size of litter is a character transmitted from mother to daughter. They recognize the smallness of the coefficient, but believe the indications of inheritance are large enough to provide a basis for selection. In studying fertility inheritance Pearson and Lee (18) obtained practically similar coefficients with the human race and the thoroughbred horse. The range of correlation was 0.0418 to 0.213; hence, they conclude that fertility is certainly and markedly inherited.

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<sup>1</sup> Paper No. 1 from the Laboratory of Animal Technology, Kansas Experiment Station.

<sup>2</sup> Reference is made by number to "Literature cited," p. 1159-1160.

Rommel and Phillips (24) studied the inheritance only through the female line, taking no account of a possible influence of the male. George (6) correlated the size of litter with that of the paternal and maternal grandams, respectively. Only 296 litters were involved in his populations; hence, his probable errors were large. But in the dam and daughter comparisons he approximated very closely the result obtained by Rommel and Phillips. His four coefficients follow:

Daughter and dam.....	0.0615±0.0390
Dam and grandam. ....	.1147±.0343
Daughter and maternal grandam.....	.0025±.0392
Daughter and paternal grandam.....	.0508±.0392

None of these correlations are three times as large as their probable error; hence, none are really significant.

Simpson (25) approached the problem from a Mendelian standpoint by crossing a wild German Schwarzwald boar to a young Tamworth sow. The Schwarzwald normally averages 4 pigs to the litter, the Tamworth about 11. The particular sow used was farrowed in a litter of 12 pigs, and to the stint of the wild boar farrowed 9 pigs. In the  $F_1$  generation three females were bred, one to a litter mate and the other two to sires unnamed. The first sow produced 4 pigs, the others 4 and 6, respectively, all in their first litters. The sow producing the 6-pig brood was later served by a pure Schwarzwald boar and farrowed 7 pigs, being apparently constant for that degree of fertility. One of the sows from the brood of 6 gave birth to 12 pigs when mated to a pure Tamworth male. The evidence for a segregation of fecundity factors seems fairly clear, although the numbers are small.

#### NONGENETIC FACTORS AFFECTING FERTILITY

External factors play a great part in the realization of the inborn hereditary capacity for reproduction. Marshall (12, 13) discusses at length the relation between season and productivity, while the sterility of wild animals in captivity or of domestic animals transferred to vastly different altitudes is proverbial. Marshall and Evvard (4, 5) have both studied the effect of "flushing" in sheep, and Evvard has conducted some very exhaustive investigations into the relation of the various compounds of nutrition to litter size in swine. Using the rate of gain at breeding time in gilts<sup>1</sup> as an indication of the state of nutrition, Evvard has found as much as an average difference of two pigs per litter in favor of the best gainers in each experimental lot, when compared with the poorest gainers. Protein, added to a nitrogen-deficient ration (corn alone) produced a marked rise in the fertility of gilts and a medium rise in the fertility of older sows.

Many stockmen believe that overfatness diminishes fecundity. There may be both a physical obstruction of the reproductive organs due to

<sup>1</sup> A gilt is a young sow intended for breeding purposes. The term is usually applied only until the first litter is produced, although it is sometimes extended throughout the suckling period.

fat and an adipose degeneration of the sex glands. Whether these are really causes of decreased fertility is doubtful, since the best evidence shows them to be symptoms of reproductive derangement.

Overfatness occurs frequently as a result of disturbances in the metabolism, due to loss of secretion from several of the ductless glands, the sex gland being here included. Castrating or spaying are known to promote obesity; hence, it is quite reasonable to assume that if testicular or ovarian derangement first occurs, then fat deposition will follow. Overfatness would thus merely indicate and not initiate reduced fecundity.

Market hog raisers usually believe that pure-bred hogs are deteriorating in prolificacy, in line with the common idea that inbreeding ultimately results in barrenness. Bitting, in 1898 (1), investigated the average size of the first 200 litters and the last 200 litters recorded at that time in the herdbooks of the Berkshire, Ohio Poland-China, Standard Poland-China, and Improved Chester White registry associations and found that during the period in which registration had taken place the Berkshires had decreased 0.19 pig per litter, the Poland-China had increased 0.225 pig, and the Chester White had increased 0.1 pig. Rommel (22) investigated the same point for a period of 20 years in books of the American and Ohio Poland-China associations, comparing the average size of litter for the first 5 years with the average for the last 5. The increase was 0.62 pig per litter among the American Poland-Chinas and 0.43 pig per litter in the Ohio strain. A similar study by Rommel (22) on the Duroc-Jersey covering over 15 years showed an increase of 0.57 pig. The changes which have occurred here are manifestly opposed to the idea that purity of blood lines diminishes fertility. On the other hand, the purity of blood can not be credited with the increase, since a constant selection for large litters has taken place, although an increased homozygosis for prolificacy might come about gradually with years of such mass selection as ordinary stock breeding involves.

Hammond (8) has shown that ova may be lost either before or after fertilization; and, still more important, he has discovered that a relatively high percentage may atrophy during the earlier stages of embryonic growth. Lewis (9) indicated that there may be morphological interferences with reproduction, so that fertility may be decreased. He found that the sperm cells of the boar are practically all dead after being in the uterus for 48 hours, which would, of course, result in a reduced fertility. Lewis's results on the viability of sperm differ from those of Dührssen (11), who observed living sperms in the Fallopian tubes of a woman patient three weeks after copulation had taken place. The importance of this question is probably confined to individual cases.

Certain relatively extraneous characters are popularly supposed to be correlated with high fertility. Many farmers believe that "big type" or "cold blooded" hogs farrow larger litters than "hot blooded," or

that "Spotted Poland-Chinas" are far more fecund than ordinary strains. Swine judges commonly consider long-bodied sows more prolific than their chubbier mates. A comparison of 1,000 litters of "large type" Poland-Chinas with 1,100 litters of "small type" showed no significant difference in fertility. The mean for the "large type" was  $7.854 \pm 0.0456$ , and for the "small type" was  $7.896 \pm 0.0436$ . Furthermore, the standard deviation of the two groups was almost exactly the same, being  $2.142 \pm 0.0323$  for the former and  $2.146 \pm 0.0309$  for the latter. The writers have never seen more than isolated instances brought forward to confirm the popular ideas on this subject and feel that the bulk of such beliefs have resulted from mere advertising schemes.

Breed certainly has its influence. Biting (1) has averaged the litter sizes for 400 Berkshires, 1,086 Poland-Chinas, and 600 Chester Whites, with the following results:

Berkshire.....	8.22 pigs per litter.
Poland-China.....	7.45 pigs per litter.
Chester White.....	8.96 pigs per litter.

Surface (26) computed the means and standard deviations in the 54,515 litters of Poland-Chinas and the 21,652 litters of Duroc-Jerseys studied by Rommel (22). His constants follow:

	Mean.	Standard deviation.
Poland-China.....	$7.435 \pm 0.01$	$2.038 \pm 0.013$
Duroc-Jersey.....	$9.337 \pm .021$	$2.427 \pm .016$

The large numbers here involved undoubtedly prove that real breed differences in fertility exist.

Pearl (15, 16) found the number of mammae to be correlated positively with the number at a birth when different species are compared, but the coefficient is very low within the species. Parker and Bullard (14) correlated the same characters in 1,000 litters of swine and obtained a coefficient of  $0.0035 \pm 0.0124$ . The senior author<sup>1</sup> treated the same point in 170 litters of which he had made genetic studies and obtained a coefficient of  $-0.0059 \pm 0.0517$ .

These figures certainly demonstrate that the number of mammae in swine is not related to fertility; in fact, nothing so far discussed presents reliable external characters on which fertility selections can be made. Apparently fecundity has as profound a genetic as physiologic basis.

#### VALUE OF HERDBOOK DATA

There is now on record an immense mass of data relating to fertility inheritance in swine, in the volumes of the different breed registry associations. In addition to the name and number of the animal, its parents, breeder, etc., the size of litter in which it was farrowed is usually stated.

<sup>1</sup> Unpublished data.

This furnishes opportunity to link together any desired number of generations.

In treating such data, the degree of confidence which can be placed in the figure for litter size must be considered. Its accuracy depends on the carefulness and honesty of the breeder, the accuracy of the clerks in the registry office, and the freedom from typographical errors in the printing of the volume. The matter of personal integrity can be accepted to a high degree, for fortunately the majority of breeders are quite reliable. Whenever falsification wittingly occurs, the tendency is to raise the number per litter; but, owing to the publicity involved in pure-bred breeding as well as the personality invested in breeding animals due to the registry systems, it is doubtful if litter sizes are ever exaggerated by more than one or two pigs.

Investigations in color discrepancies, mistakes in parentage, etc., have shown that about 2 per cent of errors are involved in the work of registry-office clerks and in printing. Some associations are more careful than others, but, of course, none are absolutely free from errors. Unfortunately swine books show a relatively greater number of mistakes than do those published by breeders of some of the other classes of live stock.

However, assuming, as has been done, that the bulk of the records can be accepted, there still remains a question as to their genetic value. It is doubtful whether a sow will ever exceed her hereditary possibilities in number per litter, but there are many forces that may cause her to fall short of that number. Lack of proper nutrition, failure to have all ova released or fertilized, loss of ova, atrophy of fertilized ova or embryos, and disease may all operate against the complete realization of the hereditary make-up. The age at which a sow farrows, the number of litters she has per year, and certain other environmental conditions may also reduce the litter size. It is interesting to observe that this source of error operates in a compensating direction to that of record falsification, when such exists, and in the end the two may counterbalance, although these physiological and pathological factors operate more often than does the misrepresentation of litter numbers.

After admitting all of these sources of error, but hoping that enough records are made under natural conditions to give the figures an investigational value, there still remains the big question of the geneticist, Does the somatic expression of the character indicate the germinal (zygotic) condition of the individual? In other words, Does the fact that a pig is farrowed in a litter of eight indicate that it will transmit a tendency to produce litters of eight? The answer very evidently is No, and the greater the degree of outcrossing in the ancestral lines, the less reliable an index of heredity the size of litter is. Yet it is the only single index obtainable in the study of herdbook records; so for the present it will have to be accepted for what it is worth.

## ADVANTAGES OF LITTER SIZE INHERITANCE STUDIES

Accepting the figures for litter size as reasonably representative of the hereditary constitution, there are a number of reasons that make them desirable material for inheritance studies. The most important of these is the fact that the male mated to a female probably does not affect the number at a birth. Instead the size of litters a sow produces represents the segregation of the tendencies transmitted to her by her father and mother. Suppose a sow produces a litter of four pigs and is herself from a litter of seven, the seven does not determine in any way the four, but instead the segregation of some tendency transmitted by her sire or dam is represented. The only check available on this tendency in her sire is the size of litter in which he was farrowed, while the same holds for the dam, except that her own breeding performance may give an additional idea.

## METHOD OF RECORDING THE DATA

The data on the animals studied were recorded as follows, the figures representing the size of litters in which the individuals were farrowed:

		Grandsire
		4
Animal	Dam	
4	7	Grandam
		9

The size of litters produced by sows whose sires came from litters of four and whose dams came from litters of seven should give an idea (through the variations recorded) of the hereditary factors involved. It is admissible that all grandams or all grandsires farrowed in the same size of litters may be different in hereditary make-up, but there should be enough individuals alike to make the frequency curves at least suggestive. For convenience, the grandparental generation will be lettered "P," the parental generation " $F_1$ ," and the filial generation " $F_2$ ," although it is clearly to be understood that this notation does not have the regular Mendelian significance.

## DEVIATIONS PER GENERATION

The mean size of 1,770 litters in the P generation was  $7.84 \pm 0.3494$ . The standard deviation was  $2.18 \pm 0.2461$ . This gives a coefficient of variability of 27.80 for this generation.

The mean size of 885 litters for the  $F_1$  generation was  $7.82 \pm 0.4897$ . The corresponding standard deviation was  $2.16 \pm 0.3462$ . The coefficient of variability here involved was 27.60, practically the same as that of the grandparental generation.

The mean size of 885 litters in the  $F_2$  generation was  $7.91 \pm 0.4965$ , while the deviation was  $2.19 \pm 0.3511$ , giving a coefficient of variability of 27.55. (See Table I.)

TABLE I.—*Deviation in size of litters in swine*

Generation.	Number of litters.	Mean.	Standard deviation.	Coefficient of variability.
P.....	1,770	7.84 ± 0.3494	2.18 ± 0.2461	27.80
F <sub>1</sub> .....	885	7.82 ± .4897	2.16 ± .3462	27.60
F <sub>2</sub> .....	885	7.91 ± .4965	2.19 ± .3511	27.55

The mean litter size is quite constant from generation to generation, and furthermore quite close to that obtained by Surface (26) for the breed in general. If anything of Mendelism is involved here, it is not revealed by this method of treatment, for the standard deviation is so nearly the same for each of the generations involved as to give no hint of segregation. In fact, the coefficients of variability would indicate a slowly increasing degree of homozygosis.

Two interpretations may be placed on these figures. The animals studied are either practically constant from a zygotic standpoint, and the variations in litter size are due to environmental treatment, or else there is so much heterozygosis present in the grandparents that the parents are as much F<sub>2</sub> as F<sub>1</sub> in hereditary make-up. For the present the writers are going to use the latter interpretation, as there is no evidence at hand to support a belief in the former.

TABLE II.—*Deviation in litter size of the offspring from the parental generation in swine*

## BOAR 1

Size of litter of parents.		Number of matings.	F <sub>1</sub> generation		F <sub>2</sub> generation	
Boar.	Sow.		Mean.	Standard deviation.	Mean	Standard deviation.
1	9	1	5	0	9	0
1	4	1	2	0	9	0

## BOAR 2

2	5	1	4	0	6	0
2	6	2	8 ± 1.43	3 ± 1.0117	7.5 ± 0.239	.5 ± 0.1686
2	8	2	6.5 ± .717	1.5 ± .1737	4 ± .479	1 ± .3372
2	9	1	6	0	6	0

## BOAR 3

3	4	3	7 ± 0.171	1.41 ± 0.3433	9 ± 1.704	4.32 ± 1.194
3	6	2	6.5 ± .239	.5 ± .1686	8 ± .95	2 ± .674
3	7	4	7.25 ± 1.6218	4.8 ± 1.1502	7.75 ± .8869	2.63 ± .627
3	11	1	6	0	8	0
3	10	2	10 ± .95	2 ± .674	11	0
3	14	1	11	0	6	0

TABLE II.—Deviation in litter size of the offspring from the parental generation in swine—Continued

BOAR 4						
Size of litter of parents.		Number of matings.	F <sub>1</sub> generation		F <sub>2</sub> generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
4	3	1	8	0	5	0
4	4	3	7.66 ± 1.032	2.62 ± 0.723	8 ± 0.631	1.63 ± 0.442
4	5	5	7.2 ± .9951	3.29 ± .7022	7.6 ± .5202	1.72 ± .3671
4	6	7	8 ± .2887	1.13 ± .2037	7.14 ± .2836	1.11 ± .2001
4	7	8	8.5 ± .6863	2.42 ± .408	5.87 ± .2726	1.14 ± .1922
4	8	6	7.83 ± .5390	1.95 ± .3801	6.83 ± .7712	2.79 ± .5438
4	9	6	7.16 ± .8127	2.94 ± .5731	7.33 ± .4008	1.45 ± .2826
4	10	4	7.25 ± .5092	1.51 ± .3618	6.75 ± .3844	1.14 ± .2012
4	12	1	7	0	12	0
BOAR 5						
5	3	1	6	0	12	0
5	4	4	5.75 ± 0.6407	1.92 ± 0.4543	6.5 ± 0.3743	1.11 ± 0.2654
5	5	3	7 ± .3195	.81 ± .2239	6.66 ± .6666	1.69 ± .4671
5	6	14	8 ± .3228	1.79 ± .2282	7.21 ± .1531	.85 ± .1083
5	7	12	7.91 ± .4464	2.29 ± .358	7.66 ± .3099	1.59 ± .2193
5	8	16	7.37 ± .3979	2.36 ± .2817	7.56 ± .408	2.42 ± .2889
5	9	9	8.11 ± .3844	1.71 ± .272	8.22 ± .5866	2.61 ± .415
5	10	6	9.33 ± .5943	2.15 ± .4191	8.66 ± .3759	1.36 ± .2651
5	11	3	6.66 ± .4891	1.24 ± .3427	8.33 ± .3707	.94 ± .2598
5	12	2	4	0	9	0
BOAR 6						
6	2	1	7	0	8	0
6	3	3	7.66 ± 0.8283	2.1 ± 0.5805	3.33 ± 0.6243	1.58 ± 0.4367
6	4	3	4.66 ± 1.1161	2.83 ± .7822	9.33 ± .4391	1.24 ± .3426
6	5	6	8.6 ± .6041	2 ± .4267	6.83 ± .4533	1.64 ± .3197
6	6	11	6.72 ± .4604	2.26 ± .3251	8.9 ± .4625	2.27 ± .3263
6	7	18	7.05 ± .2672	1.68 ± .1888	8.5 ± .252	1.86 ± .178
6	8	24	7.58 ± .3255	2.36 ± .2300	7.46 ± .1862	1.35 ± .1315
6	9	15	8.4 ± .0046	2.74 ± .0311	8 ± .047	2.7 ± .0314
6	10	8	6.62 ± .4657	1.71 ± .2885	7.87 ± .6075	2.54 ± .4283
6	11	5	9 ± .9151	2.32 ± .6414	5 ± .6922	2.29 ± .4887
6	12	3	7.66 ± .1853	.47 ± .1299	8.33 ± 1.136	2.88 ± .796
BOAR 7						
7	3	1	6	0	10	0
7	4	4	7.5 ± 0.8431	2.5 ± 0.5802	8.5 ± 0.1631	5 ± 0.119
7	5	7	7.71 ± .4202	1.62 ± .292	7.85 ± .6106	2.39 ± .431
7	6	17	7.25 ± .2177	1.33 ± .1541	7.58 ± .239	1.46 ± .1691
7	7	19	7.52 ± .3023	1.95 ± .2135	7.42 ± .3178	2.05 ± .2244
7	8	26	8.03 ± .3637	1.99 ± .1843	7.8 ± .2545	1.92 ± .1778
7	9	41	9.29 ± .1958	1.83 ± .1363	8.82 ± .2077	1.94 ± .1445
7	10	8	8.37 ± .5333	2.23 ± .3760	6.62 ± .4616	1.93 ± .3254
7	11	16	8.5 ± .2596	1.54 ± .1838	8.43 ± .4502	2.76 ± .3187
7	12	6	8.66 ± .5943	2.15 ± .4191	6.5 ± .6109	2.21 ± .4302
7	13	1	10	0	4	0



TABLE II.—Deviation in litter size of the offspring from the parental generation in swine—Continued

BOAR 8						
Size of litter of parents.		Number of mat-ings.	F <sub>1</sub> generation.		F <sub>2</sub> generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
8	3	7	7.28±0.1124	0.44±0.0793	7 ±0.3193	1.25±0.2254
8	4	8	8.75±.6721	2.81±.4738	7.75±.5572	2.33±.3928
8	5	11	7.33±.2634	1.91±.1861	8.27±.6377	3.13±.4501
8	6	24	6.91±.2965	2.15±.2094	7.66±.3062	2.22±.2161
8	7	29	7.86±.2394	1.91±.1692	7.82±.277	2.21±.1958
8	8	43	6.58±.2519	2.45±.1783	7.23±.2076	2.02±.1469
8	9	34	8.14±.292	2.52±.2124	8.17±.2294	1.98±.1669
8	10	10	6.7 ±.2219	1.04±.1565	8.4 ±.1462	5.37±.8083
8	11	10	7.8 ±1.024	4.8 ±.7225	8.6 ±.512	2.4 ±.3612
8	12	3	9 ±1.4061	3.55±.9895	9.66±.8415	2.04±.5639
8	13	1	9	0	10	0
BOAR 9						
9	2	1	5	0	9	0
9	3	1	5	0	7	0
9	4	6	7.83±0.4616	1.67±0.3254	7.66±0.4616	2.05±0.3996
9	5	12	7.5 ±.3898	2 ±.2758	6.91±.4482	2.3 ±.3172
9	6	7	6.71±.4444	1.74±.3138	7.14±.6065	2.35±.4238
9	7	32	7.59±.2232	1.87±.1576	8.53±.2495	2.09±.1762
9	8	26	7 ±.2663	2.01±.1861	7.5 ±.3074	2.32±.2148
9	9	35	7 ±.3172	2.95±.2377	8.71±.2158	2.10±.1693
9	10	14	8.66±.4060	2.33±.2874	7.53±.3468	1.99±.2453
9	11	8	7.75±.409	1.71±.2883	8.5 ±.526	2.2 ±.3709
9	12	4	8 ±.1686	.5 ±.119	9.25±.3709	1.1 ±.263
9	13	1	7	0	8	0
9	15	1	6	0	8	0
BOAR 10						
10	1	1	8	0	14	0
10	3	2	9 ±0.4784	1 ±0.3372	7.5 ±1.674	3.5 ±1.1803
10	4	2	9	0	7.5 ±1.674	3.5 ±1.1803
10	5	4	7 ±.4755	1.41±.3372	7.25±.4957	1.47±.3516
10	6	6	7.66±.34	1.23±.2397	8 ±.3897	1.41±.2749
10	7	16	7.93±.3591	2.13±.2542	8.18±.4991	2.96±.3533
10	8	24	8.29±.2978	2.16±.2105	8.26±.3787	2.69±.2676
10	9	11	8.9 ±.6153	3.02±.4343	8.63±.3586	1.76±.2531
10	10	14	6.78±.4128	2.29±.2919	7.57±.2777	1.54±.1963
10	11	4	8.25±.435	1.29±.3085	9.5 ±.5598	1.66±.397
10	12	6	8.5 ±.525	1.9 ±.370	8.63±.5888	2.13±.4151
10	14	1	10	0	8	0
BOAR 11						
11	3	1	9	0	10	0
11	4	1	6	0	9	0
11	5	5	6.8 ±0.0574	1.9 ±0.0405	8.2 ±0.5111	1.69 ±0.3607
11	6	6	8 ±.5749	2.08±.4054	6.33±.4644	1.68 ±.3275
11	7	12	7.33±.3004	1.54±.2124	7.85±.3804	2.007±.2705
11	8	4	8.5 ±.1686	.5 ±.1190	8.75±1.2107	3.59 ±.8586
11	9	6	8.83±.6053	2.19±.4269	8.16±.4809	1.74 ±.3391
11	10	6	7.5 ±.3434	1.25±.2422	8.66±.4646	1.69 ±.3277
11	13	1	6	0	12	0
11	15	1	9	0	9	0

TABLE II.—Deviation in litter size of the offspring from the parental generation in swine—Continued

BOAR 12						
Size of litter of parents.		Number of mat-ings.	F <sub>1</sub> generation.		F <sub>2</sub> generation.	
Boar.	Sow.		Mean.	Standard deviation.	Mean.	Standard deviation.
12	2	1	9	0	7	0
12	4	2	9.5 ± 0.238	.5 ± 0.118	8.5 ± 0.238	.5 ± 0.118
12	5	2	4 ± .956	2 ± .6745	7	0
12	6	1	8	0	10	0
12	7	5	7.6 ± .554	1.85 ± .3001	9.2 ± .399	1.32 ± .282
12	8	6	8.82 ± .384	1.39 ± .27	8.5 ± .671	2.4 ± .473
12	9	6	6.5 ± .591	2.14 ± .414	7.83 ± .387	1.4 ± .272
12	10	1	11	0	10	0
12	11	2	11	0	8.5 ± .717	1.5 ± .505
12	12	1	10	0	12	0
12	13	2	7	0	9.5 ± .717	1.5 ± .505
BOAR 13						
13	6	3	8.66 ± 0.493	1.25 ± 0.345	8.33 ± 0.185	0.47 ± 0.129
13	8	2	9.5 ± .717	1.5 ± .505	7 ± .956	2 ± .6745
13	9	2	11.5 ± .717	1.5 ± .505	9 ± .956	2 ± .6745
13	11	1	10	0	6	0
13	12	2	7.5 ± .168	.5 ± .245	10 ± .479	1 ± .337
13	13	5	9.6 ± .526	1.74 ± .371	10 ± .956	2 ± .6745
BOAR 14						
14	8	2	9	0	10.5 ± 0.239	0.25 ± 0.1686
14	9	1	9	0	11	0
14	12	1	7	0	3	0
BOAR 15						
15	8	1	12	0	8	0

## INDIVIDUAL EVIDENCES OF SEGREGATION

Table II is produced by treating the litter size as a detailed character and comparing the parental generation with offspring. The average of the F<sub>1</sub> deviations is  $1.87 \pm 0.0549$ , while the F<sub>2</sub> mean deviation is  $1.92 \pm 0.0582$ . The probable errors make these two constants overlap, so that the individual treatment when lumped seems no more significant than when the deviations per generation are considered. Yet many individual evidences of segregation exist, and many times the F<sub>2</sub> generation from a particular cross is so small in numbers that only a fragmentary view of the segregable possibilities is obtained.

While it is possible that 90 per cent of the litter sizes in these tables do not represent the exact genetic constitution, yet it is probable that in general the greater the disparity in litter sizes between the two animals in the P generation, the greater will be the expected deviations in the  $F_2$ , and the smaller the deviations in the  $F_1$  generation. The following results, Table III, are produced by tabulating the averages of the deviations on this basis.

TABLE III.—Average deviations in litter size in the  $F_1$  and  $F_2$  generations of swine

Difference in number of pigs in the two P litters	0	1	2	3	4	5	6	7	8
$F_1$ deviations . . . . .	2. 13	1. 89	1. 93	2. 04	2. 19	1. 82	1. 32	1. 12	0. 5
$F_2$ deviations . . . . .	1. 91	1. 84	2. 16	2. 10	2. 16	1. 71	1. 72	1. 98	. 5

A calculation of the probable errors involved in this table shows that only the difference between the  $F_1$  and  $F_2$  deviations where the disparity in litter size is seven pigs is large enough to be mathematically significant. The difference when the parents vary from each other by two pigs and by six pigs is on the border line between significance and nonsignificance, but the five other columns are distinctly unenlightening. Yet, if the difference of two pigs is barred, the results are what might be expected.

One criticism against the preceding method of treatment is thoroughly valid. If swine fertility depends on only one or two genetic factors, it is obvious that the point at which the difference between the two parents occurs is more important than the degree of difference. For example, if there is a physiological division point between two hereditary factors at six pigs, then a difference of two or even of four below six pigs might not be significant, while a difference of one more or one less in a litter of six or seven pigs would be thoroughly significant. An examination of the data from this point of view is now in progress, but it is probable that the key to the situation will only be discovered by breeding experiments.

#### CURVES OF LITTER FREQUENCIES

The distribution of the different sizes of litters in the three generations is given in Table IV. \*

TABLE IV.—Litter frequencies in swine

Generation.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P { Expected . . .	0. 11	1. 5	9. 8	39	108	216	324	370	324	216	108	39	9. 8	1. 5	0. 11
P { Actual . . . . .	3	9	30	80	124	198	300	362	318	162	91	59	26	6	3
$F_1$ { Expected . . . .	. 05	. 75	4. 9	19	54	108	162	185	162	108	54	19	4. 9	. 75	. 05
$F_1$ { Actual . . . . .	0	5	14	32	69	122	149	161	149	85	62	23	8	4	2
$F_2$ { Expected . . . .	. 05	. 75	4. 9	19	54	108	162	185	162	108	54	19	4. 9	. 75	. 05
$F_2$ { Actual . . . . .	0	4	17	32	63	107	154	172	135	95	59	30	11	3	3

Figures 1, 2, 3, and 4 show the curves for the litter frequencies in the three generations and indicate how close the actual numbers of litters come to the binomial curve  $(x+y)^{14}$ . It is perhaps incorrect to call the theoretical frequencies recorded in Table IV "expectations," unless it is clearly understood that they are the expectations founded on the nearest binomial. There is nothing in the inheritance to make them true expectations

from an experimental standpoint.

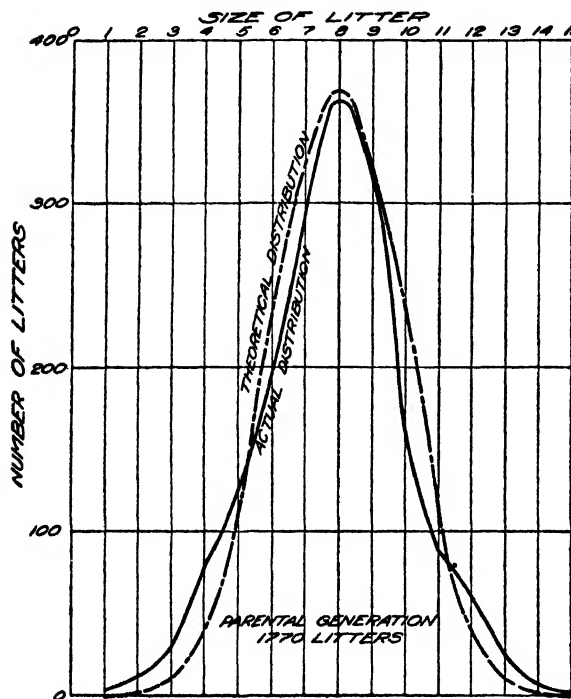


FIG. 1.—Curve of litter frequencies in the P generation of swine.

mean for each perfectly valid. The modes of these three curves are as follows:

Curve 1.....	4 pigs per litter.
Curve 2.....	8 pigs per litter.
Curve 3.....	12 pigs per litter.

It is premature to announce that these modes represent centers of deviation for genetic factors, although a casual observation of the individual data makes it seem that this condition may exist. Furthermore, the mode of curve 1 corresponds to the degree of fertility which Simpson states is characteristic of the wild hog, while the mode of curve 3 is very close to that of the Tamworth, the most fecund of domestic breeds. This indicates that the two may represent basic and improved factors for fertility, respectively, while curve 2 represents heterozygous conditions.

Before these curves can be accepted as more than merely suggestive a further analysis must be made. There is a significant deviation from expectancy in the right-hand branch of the curve of the total population, which persists even after the separation into three curves. In figure 4 this deficiency is located in the left-hand branch of curve 3, but the minus deviations may just as logically belong in the right-hand branch of curve 2, suggesting that it also may be compounded of two curves dependent on a genetic factor not disclosed thus far.

Paralleling this study some actual matings of swine have been planned and are in progress.

#### SUMMARY

(1) Fertility in swine offers favorable material for the study of quantitative inheritance, because the units of deviation are discrete.

(2) Biometric studies of litter size with mother and daughter have indicated a small degree of inheritance.

(3) Crosses of breeds having different mean

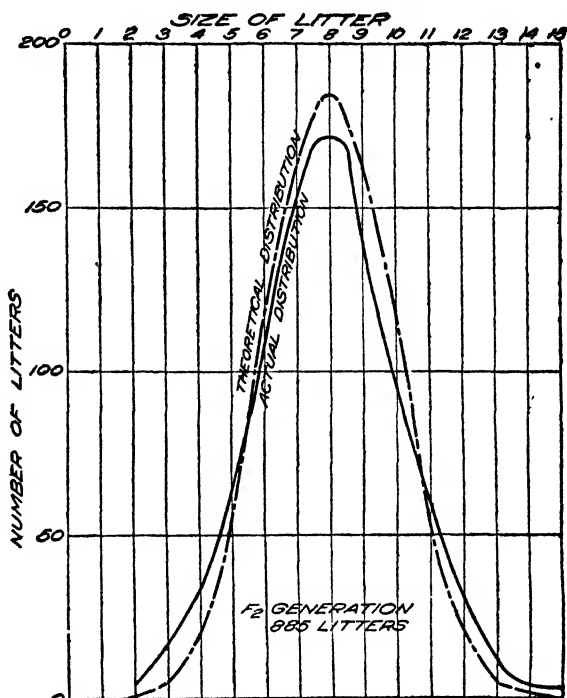


FIG. 2.—Curve of litter frequencies in the  $F_2$  generation of swine.

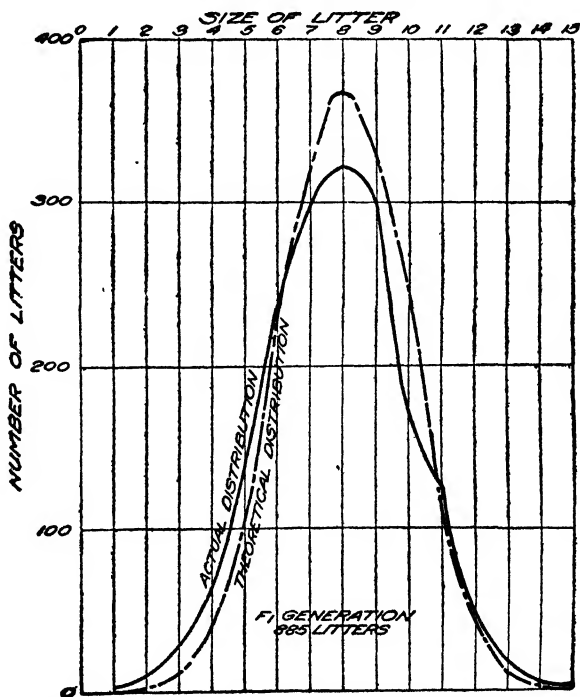


FIG. 3.—Curve of litter frequencies in the  $F_1$  generation of swine.

litter sizes have suggested that segregations of fecundity factors may take place.

(4) Numerous nongenetic factors limit the full expression of the inborn possibilities of fertility.

(5) Certain few somatic characters may be correlated either in a physiological or genetic manner with the different degrees of fecundity, but the bulk of characters usually assumed to be so related are probably entirely independent of it.

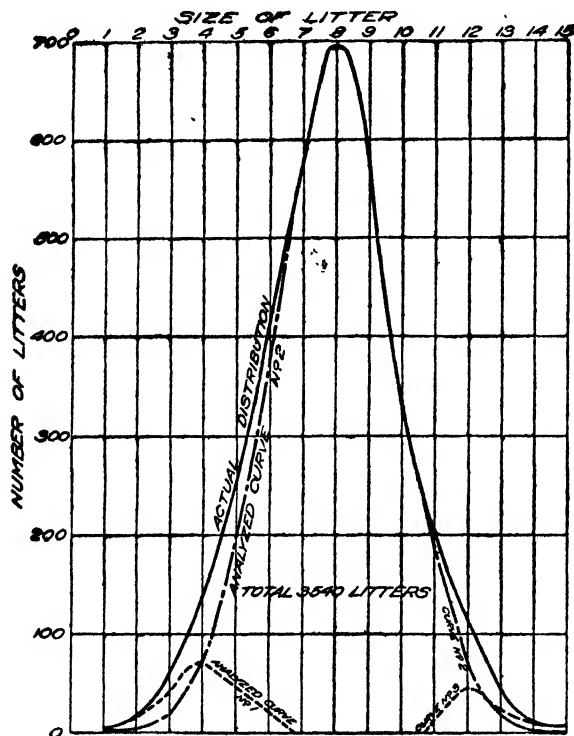


FIG. 4.—Diagram of the combined litter frequencies for the three generations of swine analyzed into its component curves.

in the litter sizes of the dams as compared with the grandparents or progeny, as would result if there were homozygous differences for fertility in the grandparents. Hence, the fertility deviations are either non-germinal or else the degree of heterozygosis is so great in the grandparents that no increased variability in the  $F_2$  generation is possible. The latter explanation is probably the correct one.

(10) The frequency curves for the 3,540 litters studied make it appear that there are at least three centers of deviation in swine fertility. These centers possibly correspond to genetic factors involved in the inheritance of fecundity.

(6) Herdbook data on the fertility of swine present sources of error, but the percentage of error is low enough to permit the statistics to be suggestive.

(7) Numerous influences exist which lower the size of litter, which sources of error may operate in a manner compensatory to those just mentioned.

(8) It is questionable whether the size of litter represents the hereditary factors transmitted, but the somatic character was perforce accepted at face value in these studies.

(9) There is no reduction in variability

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# RELATION OF GREEN MANURES TO THE FAILURE OF CERTAIN SEEDLINGS

By E. B. FRED,  
*Agricultural Bacteriologist, Agricultural Experiment Station of the  
University of Wisconsin*<sup>1</sup>

## INTRODUCTION

In a previous report it has been shown that if green manures are turned under and the soil planted immediately, a decrease in germination may result. For example, a 20-acre field, half in crimson clover (*Trifolium incarnatum*) and half in fallow, was plowed and planted to cotton (*Gossypium* spp.) (17, p. 26).<sup>2</sup> On the crimson-clover plot the cotton failed almost completely to germinate. Here and there a few crippled seedlings appeared, while on the fallowed plot normal germination occurred. Seed from the same lot was used on both plots. The green manure in some way seriously affected the germination of the cottonseed. Three weeks later the green-manure plot was again seeded to cotton. Germination at this time was perfectly normal. Apparently the harmful factor disappeared during the interval of three weeks.

A more extensive study of the substances affecting seed germination and of the factors involved was deemed advisable. The controlling idea in this investigation was a study of the effect of green manures on the germination of different seeds. In determining the percentage of germination, only those seedlings that appeared above the surface are recorded.

The amount of green manure used was determined from the following calculation: A good crop of clover should yield from 4 to 5 tons of undried green hay per acre. If 1 acre of soil 3 inches deep weighs 1,000,000 pounds, then 1 per cent of green clover is comparable to the amount employed under field conditions. Except in rare cases this amount of green manure was used in all of the laboratory studies. The green plant tissue was cut just before blooms began to form, finely chopped, and mixed thoroughly with Miami silt loam soil from the Experiment Station farm. The soil moisture was maintained at 50 per cent saturation. All tests of germination are recorded in percentages. Photographs were made of the young seedlings two weeks after planting.

## EFFECT OF GREEN MANURES ON THE GERMINATION OF VARIOUS SEEDS

Since it has been shown that seeds of different plants vary widely in chemical composition, it is very probable that they will react differently toward green manures. This experiment was planned to test the effect

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<sup>1</sup> Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Reference is made by number to "Literature cited," p. 1175-1176.

of decomposing plant tissue on the germination of buckwheat, castor beans, corn, crimson clover, flax, hemp, lupines, mustard, oats, peanuts, soybeans, sunflower, and wheat. The percentage composition of these seeds is given in Table I.

TABLE I.—The percentage composition of various seeds (11, 20)

Name.	Fat.	Crude protein.	Nitrogen-free extract.	Crude fiber.	Ash.
Castor bean ( <i>Ricinus communis</i> )	51. 37	18. 75	1. 5	18. 1	3. 1
Peanut ( <i>Arachis hypogaea</i> ) . . . .	45	25	18	.....	2 to 5
Flax ( <i>Linum usitatissimum</i> ) . . . .	33. 7	22. 6	23. 2	7. 1	4. 3
Hemp ( <i>Cannabis sativa</i> ) . . . . .	32. 58	18. 23	21. 06	14. 97	4. 24
White mustard ( <i>Brassica alba</i> ) . .	29. 66	27. 59	20. 83	10. 27	4. 47
Sunflower ( <i>Helianthus annuus</i> ) . .	28. 79	16. 3	17. 28	27. 9	3. 3
Cotton ( <i>Gossypium herbaceum</i> ) . .	20. 86	19. 69	23. 43	21. 1	3. 8
Soybean ( <i>Glycine soja</i> ) . . . . .	17. 00	35. 00	26. 00	5 to 6	4. 5
White lupine ( <i>Lupinus albus</i> ) . . .	6. 79	28. 78	33. 65	11. 92	2. 99
Oat ( <i>Avena sativa</i> ) . . . . .	5. 27	10. 25	59. 68	9. 97	3. 02
Corn ( <i>Zea mays</i> ) . . . . .	4. 5	9. 5	68. 5	2. 18	1. 6
Buckwheat ( <i>Fagopyrum tataricum</i> ) . . . . .	2. 68	11. 41	58. 79	11. 44	2. 38
Wheat ( <i>Triticum sativum</i> ) . . . .	1. 65	10. 93	70. 01	2. 12	1. 92

The seeds are grouped according to fat content; those richest in fat are given first. The marked difference in the chemical composition of various seeds is very noticeable. For instance, castor beans contain more than 50 per cent of fat, while oats contain less than 2 per cent.

According to Nobbe (16, p. 173), seeds rich in oil require more oxygen for germination than starch seeds. In Tables II, III, and IV data are presented concerning the effect of green manures on various seeds. In every case the seeds were tested under identical conditions. The figures of Table II show the effect of 1 per cent of green clover on the germination of buckwheat, corn, hemp, lupine, and sunflower.

TABLE II.—Effect of green clover on the germination of various seeds

No.	Seed.	Treatment.	Germination.		
			1 week.	2 weeks.	Relative.
			Per cent.	Per cent.	Per cent.
1	Buckwheat. . . . .	None. . . . .	75	90	100
2	do. . . . .	1 per cent clover . . . . .	90	90	100
3	Corn . . . . .	None. . . . .	100	100	100
4	do. . . . .	1 per cent clover . . . . .	95	100	100
5	Hemp. . . . .	None. . . . .	95	95	100
6	do. . . . .	1 per cent clover . . . . .	65	65	68
7	Lupine. . . . .	None. . . . .	75	80	100
8	do. . . . .	1 per cent clover . . . . .	60	60	75
9	Mustard. . . . .	None. . . . .	95	95	100
10	do. . . . .	1 per cent clover . . . . .	55	55	58
11	Sunflower . . . . .	None. . . . .	90	90	100
12	do. . . . .	1 per cent clover . . . . .	90	90	100

The average percentage of germination in duplicate pots, after one and two weeks, is recorded in Table II. The last column gives the relation between the treated and untreated seeds. A glance at the figures shows clearly that buckwheat, corn, and sunflower were not injured by green manures. On the other hand, hemp and mustard were seriously injured; the latter showed the greatest loss. Lupines are not so sensitive as mustard or hemp toward green manure, although a slight decrease in germination is noted.

As regards fat content, it will be seen that with the exception of sunflower those seeds rich in oil are the most sensitive to green manuring. The very quick germination of sunflower seed may explain their resistance to the injurious factor.

Table III presents data to show the striking difference in behavior of fat and starch seeds toward green manures. A comparison of the injury resulting from the use of green clover and green oats is made.

TABLE III.—*Effect of green clover and oats on the germination of cottonseed and wheat*

No.	Seed	Treatment	Germination			
			1 week.	2 weeks	3 weeks.	Relative.
			<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	Cotton	None.....	85	92.5	92.5	100
2	do	1 per cent of oats ...	45	65	65	70
3	do	1 per cent of clover	17.5	17.5	17.5	19
4	Wheat	None.....	95	100	100	100
5	do	1 per cent of oats.	85	90	90	90
6	do	1 per cent of clover	85	85	85	85

The germination of cotton was seriously injured by the presence of green manures; the green clover was much more harmful than oat tissue. Wheat was little affected by the use of green manure. The data confirm the results of the preceding test—that is, that seeds rich in oil are especially sensitive to green manures. It appears that the percentage of injury depends to a certain degree on the source of the plant tissue. Plate LXXXIII, figure 1, is reproduced from a photograph of cotton seedlings two weeks after planting. In order to make the seedlings more visible, a thin layer of white quartz sand was poured upon the surface of the soil.

With soybeans in place of wheat, this experiment was repeated, as shown in Table IV.

TABLE IV.—Effect of green clover and oats on the germination of cottonseed and soybeans

No.	Seed.	Treatment.	Germination.			
			1 week.	2 weeks.	3 weeks.	Relative.
			Per cent.	Per cent.	Per cent.	Per cent.
1	Cotton . . .	None . . . . .	95	100	100	100
2	....do . . .	1 per cent of oats . . . . .	35	35	35	35
3	....do . . .	1 per cent of clover . . . . .	.....	10	10	10
4	Soybean . . .	None . . . . .	100	100	100	100
5	....do . . .	1 per cent of oats . . . . .	40	40	40	40
6	....do . . .	1 per cent of clover . . . . .	30	60	60	60

Here it was again found that the oil seeds are very sensitive to green-manuring. Soybeans are more resistant to this injury than cotton.

As regards the source of the green manure, the results of numerous tests indicate that clover causes a greater loss than oat tissue. An exception to this is found with soybeans (Table IV). No satisfactory explanation has been found for the different action of these two substances. The average of three total-nitrogen analyses shows that clover contains 80.27 per cent of moisture and 4.8 per cent of protein (dry basis). The oats contained 82 per cent of moisture and 3.96 per cent of protein. Chemical analyses fail to disclose any very striking differences between the clover and oat tissue. Indeed, the protein content is nearly the same in both substances. It is possible that the nitrogen of legumes is more available than that of nonlegumes (14). It was noticed repeatedly that clover tissue decomposes more rapidly than oat tissue.

EFFECT OF TIME OF PLANTING AND QUANTITY OF GREEN MANURE ON THE GERMINATION OF COTTON SEED

Ten half-gallon jars were filled with soil and treated as shown in Table V.

TABLE V.—Effect of time of planting and quantity of clover on the germination of cottonseed

No.	Treatment	Germination.							
		Planted immediately.				Planted two weeks later.			
		1 week.	2 weeks.	3 weeks.	Relative.	1 week.	2 weeks.	3 weeks.	Relative.
1	None. . . . .	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
2	0.25 per cent of clover . .	90	90	90	100	90	95	95	100
3	0.5 per cent of clover . .	60	60	60	66	90	95	95	100
4	1.0 per cent of clover . .	50	50	50	55	80	95	95	100
5	2.0 per cent of clover . .	35	35	35	38	100	100	100	100
6	3.0 per cent of clover . .	.....	.....	.....	.....	75	85	85	89
						70	85	85	89

From the data of this experiment it is very evident that the serious injury caused by green manures is only temporary. Two weeks after the green manure was turned under, the conditions that affect seed germination disappeared. Aside from the temporary nature of the injurious agent, it will be seen that the percentage of injury is fairly proportionate to the amount of green clover used. In the presence of 0.25 per cent, the rate of germination was decreased 34 per cent, while more than 1 per cent of green manure entirely prevented germination. A comparison of the effect of green manures in different stages of decomposition on cotton germination is shown in Plate LXXXIII, figures 2 and 3.

#### FIELD EXPERIMENTS WITH GREEN MANURES

Early in the spring of 1914 a series of plot experiments with various seeds was made. For this purpose a good clover sod from the Experiment Station farm, near Madison, Wis., was chosen. This sod was divided into three equal sections: *A*, Clover; *B*, oats; and *C*, unplanted. The sections were subdivided into six plots, as shown in Table VI. Section *A* was allowed to remain in clover, while *B* and *C* were plowed, section *B* planted to oats, and *C* left without any crop. When the oats in section *B* and the clover in section *A* were partly in bloom, the soil was plowed and prepared for planting. One half of each section was planted immediately, the other half 25 days later. It was arranged to study the effect of clover and oat tissue on the germination of cotton, corn, hemp, oats, and soybeans. The same weight of seed was planted in each plot. The results of this series of tests are given in Tables VI and VII.

TABLE VI.—*Effect of green clover on the germination of various seeds*

No.	Seed.	Planted immediately after turning under.				Germination of seed planted 25 days after turning under.	
		With clover.		Unplanted.		With clover.	Unplanted.
		Seed germination.	Weight.	Seed germination.	Weight.		
			<i>Pounds.</i>		<i>Pounds.</i>		
1	Cotton.....	60	...	91	...	190	210
2	do.....	71	...	129	...	202	218
3	Corn.....	76	21	79	27	68	75
4	Hemp.....	Few.	8	Many.	27	1,050	1,130
5	Oats.....	505	.....	474	...	Fine.	Fine.
6	Soybean.....	58	4	83	5.5	83	88

TABLE VII.—*Effect of oats on the germination of various seeds*

No.	Seed.	Germination of seed.			
		Planted immediately after turning under.		Planted 25 days after turning under.	
		With oats.	Unplanted.	With oats.	Unplanted.
1	Cotton . . . . .	100	210	134	140
2	do. . . . .	117	218	125	131
3	Corn . . . . .	62	75	72	73
4	Hemp . . . . .	450	1,130	210	320
5	Oats . . . . .	Many.	Many.	Many.	Many.
6	Soybean . . . . .	35	88	39	40

From these tables it will be seen that green manures seriously injure the germination of cotton, soybeans, and hemp, while corn and oats are not affected. The diminished germination is not confined to clover tissue, but is noted with oats. This effect of the plant tissue on germinating seeds is also observed in the weight of harvest. Unfortunately, because of climatic conditions, the cotton could not be grown to maturity. On adjoining plots, where the green manure was allowed to decompose for 25 days before planting, no injury was observed.

The field data show (1) that green manures largely prevent the germination of certain oil seeds, and (2) that the unfavorable condition is only temporary.

#### NATURE OF THE INJURIOUS AGENT

There are a number of possible causes that might account for the destructive influence of green manures on seed germination:

First, the green manure greatly increases the number and variety of micro-organisms. The organisms on the plant tissue may be harmful, or conditions proper for the development of harmful organisms may arise.

Second, the large gain in number of organisms, after the addition of green manure, results in a possible accumulation of substances toxic to germination—for example, poisonous by-products of decomposition, as alkali or acid.

Third, the rapid multiplication of micro-organisms, which results in an increased metabolism, causes soil oxygen to be consumed and carbon dioxid to be given off. Such loss in oxygen and gain in carbon dioxid might conceivably retard or prevent germination. If it is assumed that oil seeds require more oxygen for germination than starch seeds, the third supposition should apply particularly to seeds rich in fat (16, p. 173).

## EFFECT OF SOIL TYPE

In order to ascertain the relation to soil type of the agent causing a decrease in germination, a series of tests was made. Four soil types were used: Colby silt loam, Miami silt loam, Sparta acid sand, and neutral sand. The results of the first test are given in Table VIII.

TABLE VIII.—*Effect of green manure on the germination of cottonseed*

No.	Soil.	Treatment.	Germination.			Relative.
			1 week.	2 weeks.	3 weeks.	
1	Colby silt loam (acid).....	None.....	<i>Per ct.</i> 90	<i>Per ct.</i> 90	<i>Per ct.</i> 90	<i>Per ct.</i> 100
2	....do.....	1 per cent of clover	35	45	50	55
3	Miami silt loam.....	None.....	75	75	75	100
4	....do.....	1 per cent of clover	35	35	35	50
5	Miami silt loam, half sand....	None.....	95	95	95	100
6	....do.....	1 per cent of clover	45	45	45	50
7	Sand.....	None.....	80	80	80	100
8	....do.....	1 per cent of clover	90	90	90	112
9	Sparta acid sand.....	None.....	80	80	85	100
10	....do.....	1 per cent of clover	70	70	70	82

For the purpose of securing variation in texture, dilutions with Miami soil and quartz sand were made. From the data obtained, it seems that the property of reducing seed germination is common to both silt loams, but is absent or almost inactive in the sands. Since the relative decrease in germination is approximately the same with Miami or Colby silt loam, it appears that soil reaction is not one of the controlling factors. In neutral or acid sand no decided injury was noted. The results of a second series of tests confirm the above statement. Just why sandy soil should prove less efficient than the loams is not evident from the data, unless it is due to the absence of the injurious factor.

## EFFECT OF POSITION OF GREEN MANURE

It was arranged to study the effect on seed germination of plant tissue at different depths. Green clover was added at the rate of 1 per cent. The results secured were as follows: When the green manure was placed in the bottom of the jar, 80 per cent of cotton germinated; in the middle, none germinated; on top, 10 per cent germinated. It is evident that green clover must be in close contact with the seed in order to be effective. This may be shown by wrapping cotton seeds with clover leaves. One or two clover leaves greatly injured seed germination. Plate LXXXIII, figure 4, shows the effect of position of green manure on seed germination.

## EFFECT OF INCREASED AERATION

In view of the different action of green manures in compact and open soils, it was decided to make a series of tests under conditions that tend to remove gaseous substances. For this purpose, specially designed jars with openings in their bottoms were employed. By means of a glass tube connected with the bottoms of the jars, air was forced through the soil. In these tests air was allowed to pass through the soil for 20 to 30 minutes every day. A comparison of germination in the aerated and unaerated soils failed to show any difference. Change in soil air did not lessen the injury.

## EFFECT OF TEMPERATURE

It is a well-known fact that slight changes in temperature often greatly increase or decrease the growth of micro-organisms. Accordingly a test was made with three variations in temperature.

TABLE IX.—*Effect of temperature on germination of cottonseed*

No.	Treatment.	Temperature.	Germination.		Relative.
			4 days.	8 days.	
		° C.	Per cent.	Per cent.	Per cent.
1	None .....	25	85	85	100
2	1 per cent of clover .....	25	55	55	64
3	None .....	30	95	95	100
4	1 per cent of clover .....	30	35	35	36
5	None .....	37	100	100	100
6	1 per cent of clover .....	37	80	80	80

About 30° C. seems to give the greatest injury; lower or higher temperatures fail to cause so great a decrease in germination.

## EFFECT OF CERTAIN DECOMPOSITION PRODUCTS

In the decomposition of plant tissue many substances are liberated—e. g., ammonia and carbon dioxide. The relation of ammonium hydroxid to seed germination has been studied by Bokorny (3; 4, p. 37). He found that small quantities of ammonium hydroxid, 0.02 per cent, greatly retarded the germination of cress. It seems that the active protein of the cell is very sensitive to ammonia.

## AMMONIUM HYDROXID

A series of tests was made using from 0.1 to 0.01 per cent of ammonium hydroxid. Four different seeds, cotton, corn, soybeans, and wheat, were allowed to germinate between cloths saturated with the varying concentrations of ammonium hydroxid. It was found that 0.05 or 0.01 per cent proved injurious, while 0.1 per cent prohibited all germination.



Since it was established that ammonia is harmful to seed germination, another test was carried out to study the ammonia produced by micro-organisms. The results of this study are shown in Table X.

TABLE X.—*Effect of sugar and of clover on ammonification*

Time in 2-day intervals.	Ammonia nitrogen in 100 gm. of soil.		
	No treat- ment.	1 per cent of sugar added	1 per cent of clover added.
	Mgm.	Mgm.	Mgm.
1. . . . .	1. 98	2. 0	3. 3
2. . . . .		2. 1	4. 3
3. . . . .		1. 96	2. 8
4. . . . .		1. 4	2. 4
5. . . . .		1. 4	2. 5
6. . . . .	1. 90	2. 5	2. 6
Total . . . . .		11. 36	17. 9

Since ammonia formation is largely a product of bacterial action, it was thought that sugar or green manure would cause an enormous increase in this substance. The data of Table X show a slight gain in ammonia in the treated soils, but the amount is far too small to affect germination seriously

## CARBON DIOXID

It was found that carbon dioxide, when added in large quantities, retards germination but does not cause the seeds to decay. As soon as the carbon dioxide is removed, germination proceeds in a normal manner. In Table XI is given the periodic evolution of carbon dioxide from soil treated with 1 per cent of sugar and 1 per cent of clover.

TABLE XI.—*Effect of sugar and clover on carbon-dioxide evolution*

Time in days.	Carbon dioxide in 100 gm. of soil		
	No treatment.	1 per cent of sugar added.	1 per cent of clover added.
	Mgm.	Mgm.	Mgm.
1. . . . .	4. 62	22. 0	16. 02
2. . . . .	6. 82	17. 2	12. 7
3. . . . .	9. 46	36. 52	22. 0
4. . . . .	7. 21	37. 84	22. 75
5. . . . .	7. 57	33. 97	22. 7
6. . . . .	7. 74	29. 35	24. 2
7. . . . .	7. 65	26. 40	24. 42
8. . . . .	9. 68	25. 30	22. 22
Total . . . . .	60. 75	228. 58	167. 01

From the data in this table it is evident that the amount of carbon dioxide evolved in the presence of sugar or clover is far too small to exert a marked effect on germinating seeds.

#### CALCIUM CARBONATE

It is well known that free acids greatly retard or prohibit germination (3; 4, p. 37). Aside from the direct effect on seeds, an acid reaction may favor the growth of injurious micro-organisms. Accordingly, two series of tests were made, using a neutral and an acid soil with varying amounts of limestone ( $\text{CaCO}_3$ ). The results of the first test are given in Table XII.

TABLE XII.—*Effect of green clover and calcium carbonate on the germination of cottonseed*

No.	Treatment.	Germination.			
		1 week.	2 weeks.	3 weeks.	Relative.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	None.....	85	85	85	100
2	1 per cent of clover.....	55	55	55	64
3	1 per cent of clover, 0.1 per cent of calcium carbonate.....	35	.....	.....	40
4	1 per cent of clover, 0.2 per cent of calcium carbonate.....	15	.....	.....	17
5	1 per cent of clover, 0.5 per cent of calcium carbonate.....	15	.....	.....	17
6	1 per cent of clover, 1.0 per cent of calcium carbonate.....	10	.....	.....	11

The data show clearly that limestone in concentrations of from 0.1 to 1 per cent seriously injured the germination of cotton. The seedlings from limed soils died during the first or second week. A second test, similar to the above, was carried out, using acid soil. Here again calcium carbonate seemed to stimulate the injurious factor.

#### EFFECT OF HEAT

The results of previous tests indicate very strongly the biological nature of the factor injurious to germination. For example, reduced germination is largely associated with the first stages of decomposition. Second, the data seem to exclude the possibility of harmful gaseous products. It is conceivable that in the early stages of decomposition green tissue is favorable to the growth of certain organisms injurious to germination. Accordingly, a series of experiments were made in which the amount and form of green manure applied, the seed, and the biological factors were modified. From 1.5 to 3 per cent of green manure was added. To remove the biological factor, the jars and contents were sterilized in the autoclave at 15 pounds' pressure for two hours. The results of this study were recorded by photographs. Reading from left to right (Pl. LXXXIV, fig. 6), the jars were treated as follows: A, none,

unsterilized; *B*, 1.5 per cent of green manure, sterilized; *C*, 1.5 per cent of green manure, unsterilized; *D*, 3 per cent of green manure, sterilized; *E*, 3 per cent of green manure, unsterilized. The soil shown in the pots in Plate LXXXIII, figure 5, was treated with green oats, in Plate LXXXIV, figure 6, with green clover. Since the corn and wheat did not show any injury, these illustrations were not reproduced. The data from cotton, clover, and flax are presented in Plate LXXXIV, figures 1, 2, 3, 4, and 5. A glance at the seedlings in the sterilized soil shows conclusively that heat removes or renders inactive the harmful factor. The percentage germination of all crops in the sterilized green-manure soil was equal to that of the untreated controls. Apparently, sterilization has in some way prevented any injury from green-manuring. This is true with 1.5 or 3 per cent of green manure. When repeated, the same results were obtained. These data are given in Table XIII. All of the results point to an injurious agent of biological nature.

TABLE XIII.—*Effect of heat on the germination of cottonseed*

Letter.	Treatment	Germination			Relative.
		1 week	2 weeks	3 weeks	
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
A	None . . . . .	95	100	100	100
B	Sterilized . . . . .	85	85	85	85
C	1 per cent of clover . . . . .	10	10	10	10
D	1 per cent of clover sterilized . . . . .	80	80	80	80
E	1 per cent of oats . . . . .	35	35	35	35
F	1 per cent of oats sterilized . . . . .	85	90	90	90

#### SOURCE OF INJURIOUS AGENT

When portions of diseased seedlings are used to inoculate sterilized green-manured soil, the germination of oil seeds is greatly reduced. Numerous tests show that the harmful agent is readily transferred. From the data it must be concluded that the injury to seed germination is biological, probably due to bacteria or fungi. To study the nature of the agent, a series of tests was made with different micro-organisms.

#### EFFECT OF BACTERIA

In this series of tests bacteria from seed, from green manure, and from soil were studied. From the nature of the seed coat of cotton it is no doubt very rich in a number of bacteria. According to plate counts, the number of micro-organisms on cottonseed is over 122,000 per gram, or an average of nearly 11,000 organisms to one seed. A comparison of the germination of cottonseed free of bacteria and with bacteria, in unsterilized green-manured soil, did not disclose any difference in germination. The bacteria were removed (2) by exposing the seed to the action of hot mercuric chlorid ( $\text{HgCl}_2$ ) or concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ). The use of sulphuric acid offers an easy and satisfactory method of

removing micro-organisms from cottonseed. The seeds were placed in a large glass-stoppered bottle containing concentrated sulphuric acid and glass beads. After shaking for two minutes, the seeds were removed with a platinum loop and washed in boiled water. From the data it seems that infection is from some source other than the seed.

It has been shown repeatedly that the addition of green manure to soil is followed by an enormous increase in the number of bacteria. Aside from the increase in bacterial food, the green manure carries with it a great number of bacteria (6, 8, 21). Tests with bacteria-free green manures failed to eliminate the injury.

About 16 pure cultures of bacteria were isolated from diseased seeds and green-manured soil. In order to test the effect of these various micro-organisms on germination, sterilized green-manured soil was inoculated with the various species of bacteria and seeded. The tests were carried out in triplicate, using bacteria-free seed of cotton, peanut, and soybeans. Here, again, bacteria failed to show any effect on the germination of oil seeds. In addition to the pure cultures used in the above experiment, a study was made with four laboratory stock cultures, *Bacillus fluorescens liquefaciens*, *B. subtilis*, *B. mesentericus vulgatus*, and *Streptothrix buccalis*. Heavy inoculations of these organisms did not injure the germination of cottonseed or soybeans. This agrees with the results of earlier workers (12, 13, 15, 18)—that is, bacteria grown on rich nitrogenous media do not injure seed germination. An exception to this is noted with cracked or injured seeds.

#### EFFECT OF FUNGI

From a study of tests carried out with various combinations of sterilized soil, green manure, and seeds free of micro-organisms, it was found that the harmful factor occurs chiefly in soil. The data in Table XIV show very conclusively the position of injury.

TABLE XIV.—Effect of fungi on the germination of cottonseed

No.	Treatment.	Germination.			
		1 week.	2 weeks.	3 weeks.	Relative.
1	Sterilized soil, 1 per cent of sterilized clover.	Per cent. 20	Per cent. 70	Per cent. 70	Per cent. 100
2	Sterilized soil, 1 per cent of unsterilized clover.....	15	45	45	64
3	Unsterilized soil, 1 per cent of sterilized clover .....				
4	Unsterilized soil, 1 per cent of unsterilized clover .....				

It seems that the harmful agent is found both in soil and in plant tissue, although it is much more prevalent in soil. The results of later tests confirm this statement.

According to many investigators, fungi may injure seed germination (1, p. 30-39; 7, 12, 15). For example, Muth (15) found *Aspergillus niger* harmful to the germination of various seeds, while Atkinson (1, p. 30-39) and Bolley (5, p. 25-27) report a destruction of cotton and flax seedlings by species of *Rhizoctonia* and *Fusarium*.

Since it is established that certain soil fungi are injurious to very young seedlings, the question arises as to the occurrence and growth of parasitic fungi in green-manured soil. An experimental study of the occurrence of fungi in green-manured soil was made. Microscopical examinations of the diseased seeds showed the presence of many fungi on the primary root tip. Although no systematic study was made, some of the forms showed certain characteristics of the genus *Rhizoctonia* and others of the genus *Fusarium*. From portions of the diseased tissue plates were poured. In this way several species of fungi were isolated. These are described under laboratory numbers. All attempts to secure a pure culture of any species of *Rhizoctonia* failed. The various fungi were used to inoculate large tubes and jars of sterilized green-manured soil. The inoculated soil was planted to bacteria-free cottonseed and soybeans. In the soil cultures no injury to germination was noted, except with culture 1. Here from 75 to 100 per cent of the seedlings were killed. Repeated tests with this unknown culture gave similar results. No injury to corn and wheat was noted from inoculations of culture 1, while soybeans and cotton were quickly destroyed.

Since the diseased root tips showed the presence of a *Rhizoctonia*-like fungus, it was arranged to study the effect of certain species of *Rhizoctonia* isolated from other sources. Two strains were employed—one isolated from potatoes, the other from alfalfa. The potato culture was secured from the Department of Plant Pathology of the Wisconsin Experiment Station; the alfalfa culture was supplied by Mr. Fred Jones, of the University of Wisconsin. Table XV gives the results of this test.

TABLE XV.—*Effect of Rhizoctonia spp. on the germination of cottonseed*

No.	Treatment and inoculum.	Germination			Relative.
		1 week.	2 weeks.	3 weeks.	
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	None, sterilized. Uninoculated.....	75	80	80	100
2	1 per cent clover sterilized. Uninoculated.....	80	85	85	105
3	None, sterilized. Inoculated with <i>Rhizoctonia</i> sp. from alfalfa.....	60	70	70	86
4	1 per cent clover sterilized. Inoculated with <i>Rhizoctonia</i> sp. from alfalfa.....	.....	.....	.....	.....
5	None, sterilized. Inoculated with <i>Rhizoctonia</i> sp. from potato.....	80	80	80	100
6	1 per cent clover sterilized. Inoculated with <i>Rhizoctonia</i> sp. from potato.....	85	85	85	105

*Rhizoctonia* sp. isolated from alfalfa proved fatal to cotton seedlings. Two weeks after inoculation all of the young plants were dead. On the contrary, a species of *Rhizoctonia* from potato produced no noticeable injury to cotton seedlings. This difference in the action of the two strains of *Rhizoctonia* is very evident from Plate LXXXIII, figure 6, and the data in Table XV. A species of *Rhizoctonia* from alfalfa produced nearly the same effect on soybeans as on cotton, while the germination of corn was not affected.

A study of the optimum conditions for the growth of culture 1 and *Rhizoctonia* sp. from alfalfa showed that about 25° to 30° C. is the most favorable temperature for both of these fungi. The results of a previous study indicate that about 25° C. is the optimum temperature for the growth of the harmful factor. From the data as a whole, it seems very conclusive that the fungus of culture 1 and probably other fungi are the causative agents in the destruction of germinating seeds.

#### DESCRIPTION OF THE INJURY

Examination of the diseased seeds shows that the injurious factor probably does not attack seeds until after germination. Apparently the fungus attacks the primary root soon after germination. This occurs when the primary root is from  $\frac{1}{2}$  to 1 cm. long. The hyphæ pierce the walls of the host, entirely envelop the root, and often penetrate deep within the tissue. In the affected region the tissue loses its form, turns brown in color, and soon rots. Under the microscope these diseased seedling roots are surrounded by a dense mantle of hyphæ, which are often brown-colored.

#### RELATION OF GREEN MANURE TO INJURY OF OIL SEEDS

Although the evidence at hand does not warrant a definite conclusion, the author suggests the following as a possible explanation for the injury: The green tissue furnishes an excellent medium for the development of fungi. This is especially true in the first stages of decomposition. After one or two weeks in the soil the green manure undergoes certain changes which render it unsuited to the growth of the injurious fungi.

Just why oily seeds should be so sensitive to fungi is not known. It is possible that the oil partly changes to fatty acids in the process of germination (9, 10). According to Schmidt (19, p. 300-303), oil and fatty acids favor the growth of certain fungi. The fungus may produce a fat-splitting enzyme—for example, lipase. This offers a possible explanation for the selective action of the injurious fungi for oil seeds.

## SUMMARY

(1) Green manures may seriously injure the germination of certain seeds.

(2) This injury is brought about by the action of certain parasitic fungi.

(3) In the first stages of decomposition of green clover, numerous fungi develop. Some of these fungi are very destructive to seedlings.

(4) Oil seeds as a class are very easily damaged by fungi. Starchy seeds, on the contrary, are very resistant.

(5) Cotton seed and soybeans are examples of seeds extremely sensitive to green manuring. The germination of flax, peanuts, hemp, mustard, and clover is reduced in the presence of decomposing plant tissue, but not to as great a degree as that of cottonseed or soybeans. The germination of buckwheat, corn, oats, and wheat is not affected by green manures.

(6) The damage to oil seeds from green manures is confined largely to the first stages of decomposition. Experimental evidence shows that two weeks after green manure is added it does not cause any serious injury to the germination of oil seeds.

(7) Small applications of calcium carbonate seemed to increase the injury to germination.

(8) The rate of germination determines to a certain extent the degree of injury. Slow germination is marked by a high percentage of diseased seedlings.

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### PLATE LXXXIII

Cotton seedlings, showing the effect of green manures on their growth:

Fig. 1.—*A, B*, Control; *C, D*, 1 per cent of chopped green oats added to the soil; *E, F*, 1 per cent of chopped green clover added to the soil.

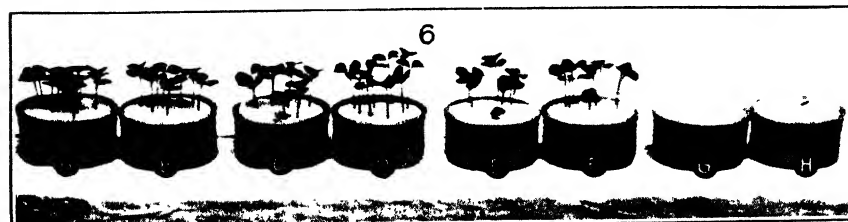
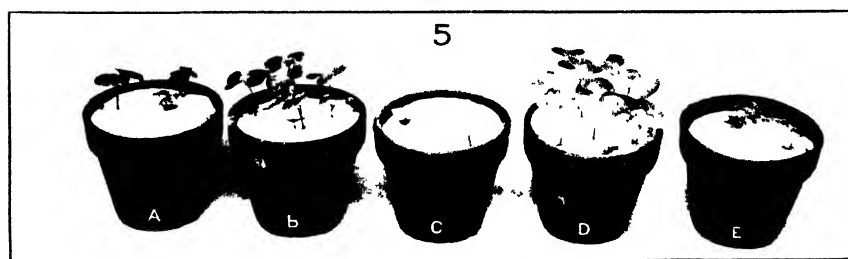
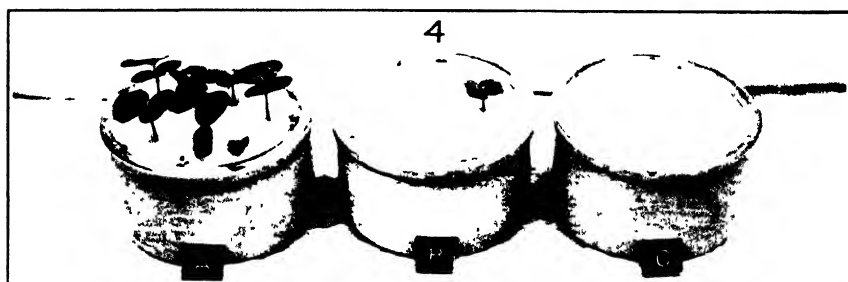
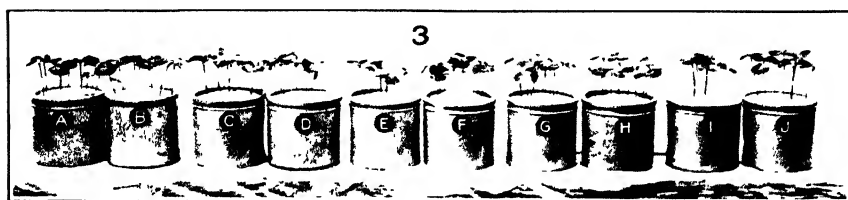
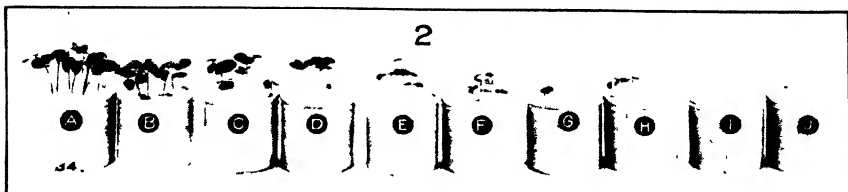
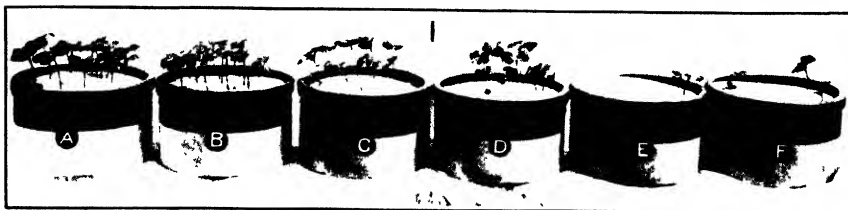
Fig. 2.—Effect of planting immediately after plowing under green manure: *A, B*, Control; *C, D*, 0.25 per cent of green manure added to the soil; *E, F*, 0.5 per cent of green manure added to the soil; *G, H*, 1 per cent of green manure added to the soil; *I, J*, 2 per cent of green manure added to the soil.

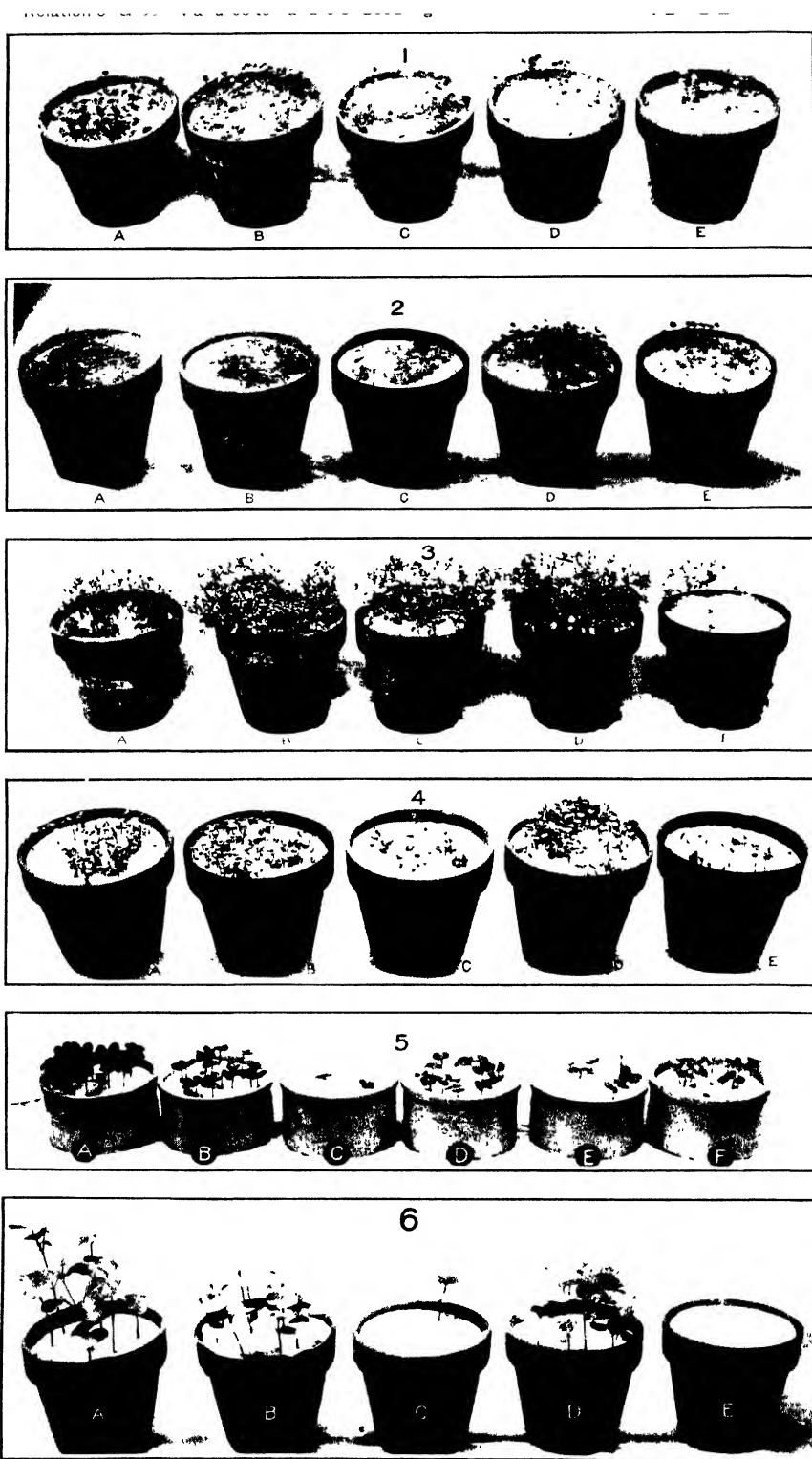
Fig. 3.—Effect of planting 2 weeks after plowing under green manure. *A, B*, Control; *C, D*, 0.25 per cent of green manure added to the soil; *E, F*, 0.5 per cent of green manure added to the soil; *G, H*, 1 per cent of green manure added to the soil; *I, J*, 2 per cent of green manure added to the soil.

Fig. 4.—Effect of the depth of green manure on germination: *A*, Green manure placed in the bottom of the pot; *B*, green manure placed at the top of the pot; *C*, green manure placed in about the middle of the pot.

Fig. 5.—Effect of sterilized and unsterilized oats used as a green manure: *A*, Control; *B*, 1.5 per cent of oats added and the mixture sterilized; *C*, 1.5 per cent of oats added without sterilization; *D*, 3 per cent of oats added and the mixture sterilized; *E*, 3 per cent of oats added without sterilization.

Fig. 6.—Effect of *Rhizoctonia* sp. on germination in the presence of green manure: *A, B*, Control; *C, D*, sterilized soil treated with green manure; *E, F*, sterilized soil inoculated with *Rhizoctonia* sp. from potatoes; *G, H*, sterilized soil treated with green manure and inoculated with *Rhizoctonia* sp. from alfalfa.





#### PLATE LXXXIV

Clover, flax, and cotton seedlings, showing the relation of green manures to germination in sterilized and unsterilized soil:

Fig. 1.—Clover: *A*, control; *B*, 1.5 per cent of chopped green oats added and the mixture sterilized; *C*, 1.5 per cent of chopped green oats added and the mixture not sterilized; *D*, 3 per cent of chopped oats added and the mixture sterilized; *E*, 3 per cent of chopped oats added and the mixture not sterilized.

Fig. 2.—Clover: *A*, control; *B*, 1.5 per cent of chopped clover added to the soil and the mixture sterilized; *C*, 1.5 per cent of chopped clover added to the soil and the mixture not sterilized; *D*, 3 per cent of chopped clover added to the soil and the mixture sterilized; *E*, 3 per cent of chopped clover added to the soil and the mixture not sterilized.

Fig. 3.—Flax: *A*, control; *B*, 1.5 per cent of chopped oats added to the soil and the mixture sterilized; *C*, 1.5 per cent of chopped oats added to the soil and the mixture not sterilized; *D*, 3 per cent of chopped oats added to the soil and the mixture sterilized; *E*, 3 per cent of chopped oats added and the mixture not sterilized.

Fig. 4.—Flax: *A*, control; *B*, 1.5 per cent of chopped clover added and the mixture sterilized; *C*, 1.5 per cent of chopped clover added to the soil and the mixture not sterilized; *D*, 3 per cent of chopped clover added to the soil and the mixture sterilized; *E*, 3 per cent of chopped clover added to the soil and the mixture not sterilized.

Fig. 5.—Cotton: *A*, control; *B*, soil sterilized; *C*, 1 per cent of chopped clover added to the soil and the mixture not sterilized; *D*, 1 per cent of chopped oats added to the soil and the mixture not sterilized; *E*, 1 per cent of chopped clover added to the soil and the mixture sterilized; *F*, 1 per cent of chopped oats added to the soil and the mixture sterilized.

Fig. 6.—Cotton: *A*, control; *B*, 1.5 per cent of chopped clover added to the soil and the mixture sterilized; *C*, 1.5 per cent of chopped clover added to the soil and the mixture not sterilized; *D*, 3 per cent of chopped clover added to the soil and the mixture sterilized; *E*, 3 per cent of chopped clover added to the soil and the mixture not sterilized.



# A NEW SPRAY NOZZLE

By C. W. WOODWORTH,

*Entomologist, Agricultural Experiment Station of the University of California*

## INTRODUCTION

A new principle has been discovered in nozzle construction whereby a flat spray can be produced with a uniform distribution of the water comparable to that of the hollow cone of spray from a cyclone nozzle. Hitherto all flat sprays have been of lenticular section, breaking up into fine mist on the sides and into relatively coarse drops in the center. It was observed that the flat spray produced by two impinging streams was at right angles to the original plane of motion of the two streams, but when the streams failed to meet squarely the plane was shifted and could, in fact, be moved through an arc of  $180^\circ$  with a very great change in the distribution of the water currents. It requires only a slight angular deviation to decrease very perceptibly the coarseness of the central drops, producing greater uniformity, and a position can be reached in which the coarsest drops are on the edge, those in the center therefore being the finest.

The principle finally discovered was that when two streams meet across half their section the resulting sheet of spray will be of practically uniform thickness throughout, occupying a plane  $45^\circ$  from the plane of the streams and finally breaking up into drops of great fineness and uniformity.

## PRODUCTION OF SPRAY

There are two causes that may act in the production of spray particles: (1) Friction, which may cause an eddy along the edge of the stream sufficient to break the surface tension and allow the small eddying masses to fly off from the column of water; and (2) divergence of the direction of motion of the particles, resulting in the thinning out of the water mass in the form of irregular sheets until the surface film finally gives way and the sheet of water is suddenly converted into drops.

Both methods may be seen in the breaking up of the stream from a simple nozzle where, from the sides of the solid column of water, very minute particles of mist are given off, while the velocity and friction are great. With decreasing velocity farther on the eddies become larger, the mist gradually becomes coarser, and, finally, as the spread of the stream makes it break up into irregular sheets of water, the size of the drops produced by the second process results in an intermingling of drops of all sizes. At first the drops are very accurately graduated,

those of the same size being produced at the same distance from the nozzle, but when the second process replaces friction as a cause of spray production, irregularity results, owing to the irregular shapes of the water sheets.

In a cyclone nozzle the stream at once diverges widely in the form of a hollow cone. Friction plays no part in the production of the spray, but the cone increases so rapidly in diameter that the liquid soon becomes a very thin sheet of unvarying thinness all the way around, and breaks into a uniformly fine mist. The uniformity may be assumed from the fact that on all sides the sheet extends an equal distance from the orifice before breaking into a spray, and experimentally can be shown to exhibit to an equally high degree both fineness and uniformity.

Figure 1 expresses in a diagrammatic form the facts shown by the photographs. The circles show the actual positions of the orifices in

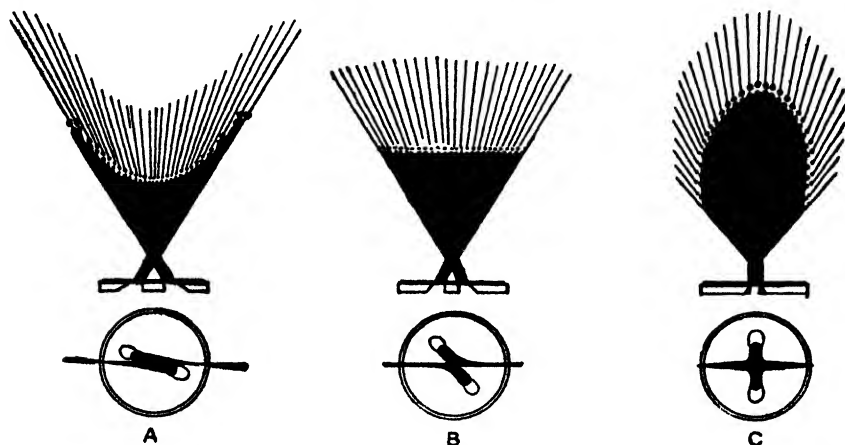


FIG. 1.—Diagram showing the characteristic differences between the three forms of impinging-stream nozzles.

each case and the black transverse marks give the effect of the impinging streams; the water remains thickest in the middle in C, thickest at the edges in A, while in B it is spread out evenly.

Above, the black portion indicates the water sheet, the sizes of the spots along the margin indicate the sizes of drops produced at these points, and the approximate velocity of the drops is shown by the length of the lines radiating from these spots.

#### SPRAYS PRODUCED BY IMPINGING STREAMS

The actual movement of the water in forming a spray through the impact of two streams is shown in Plate LXXXV and Plate LXXXVI, figure 1. It was not found practicable to secure the successive pictures with sufficient rapidity to show more than two steps in the forming spray, but by interpolating, a fairly satisfactory series was obtained. The



right-hand nozzle is of the common type where the streams impinge squarely. The middle nozzle is of the new type, but not strictly comparable with the former, since the streams come together at a broader angle, making a wider spray. Indeed, when the spray is under full pressure (Pl. LXXXVI, fig. 1) the spread is too wide, producing a lateral dribble and marginal fringe of spray. The left-hand spray is intermediate in angle and spread and gives the fish-tail effect.

The contrast is shown from the first illustration, the fish tail having thick marginal zones and the other two thick central zones, much shorter in the middle nozzle. In Plate LXXXVI, figure 1, where the spray sheets assume their normal proportions under high pressure, the large size of the white patch in the middle corresponds to the better final distribution of the spray particles. The irregularity of the spot shown on the left of this white patch is due to an irregularity in the orifice on the opposite side.

In Plate LXXXVI, figure 2, which shows the result of a sudden decrease of pressure, the character of the water sheets becomes especially evident, since they are increased greatly in size and the production of spray almost ceases.

#### ADVANTAGE OF A FLAT SPRAY

The cyclone nozzle leaves nothing to be desired in the way of fineness and uniformity of spray, but it has the disadvantage of making a ring of spray which surrounds instead of touching the object towards which the nozzle is directed. It is very difficult for one handling the nozzle to keep in mind the fact that the spray is strictly limited to the visible parts of the cone. A flat spray, on the other hand, reaches the point aimed at and is more available for treating branches of trees, for example, where the desire is to concentrate the spray on a line. For general spraying also the use of a flat spray, like the use of a flat brush for painting, gives uniform results more quickly and easily. For these reasons, while no other nozzle on the market produces a flat spray comparable in quality to the spray produced by the various types of cyclone nozzles, they are, nevertheless, more extensively used than the cyclone nozzles.

#### ADVANTAGE OF UNIFORMITY AND FINENESS

The use of nozzles of the flat type is generally acknowledged to be for the purpose of securing the flat shape of spray fan and is not a rejection of the principle that a uniformly fine spray is the most desirable. In fact, the use of these nozzles is generally associated with the use of high pressures, whereby the defects of a poor grade of nozzle are less apparent. The particular advantage of fineness is that it makes possible the even distribution of the spray material.

Fineness involves evenness. In a nozzle giving coarse drops, part of the material is in a finely divided state, and the improvement in a spray

nozzle comes through decreasing the size of all but the smallest particles and thus increasing the proportion of minute particles until, as in the cyclone nozzle, practically all of the material is in the most finely divided state and is therefore also uniform. This improvement can be produced by increasing the pressure or decreasing the size of the stream. Under the same pressure a nozzle with a large orifice gives coarser drops than a similar nozzle with a small orifice. Therefore, where a larger volume of spray is desired, it has been the practice to duplicate the nozzles rather than enlarge them, giving clusters of nozzles; but where high pressure is available, large nozzles, particularly those of the better type, may be used. With extreme pressures, such as were employed in the gipsy-moth work and in the walnut spraying in California, a nozzle of the poorest quality and rather large size has proved to be practical. In nearly all cases the desirability of fine and uniform sprays, in order to secure evenness of distribution, has been recognized. It is possible, however, that under some circumstances a driving spray may be desirable, and this can be secured only by the use of less efficient nozzles.

#### VARIATION IN FINENESS

The sizes of the smallest drops in a spray are not necessarily the same, particularly when made by the breaking up of a sheet of water. By a change in the proportions of the eddy chamber in a cyclone nozzle or by a change in spraying pressure the diameter of the cone at the point of breaking can be changed, and the drops will remain uniform, but will be of a different size than before. In the new type of nozzle here described the angle of impact and the spraying pressure exert similar effects, and a series of nozzles can be produced covering much the same range obtainable in a cyclone nozzle and distinguishable by the width and length of the fan.

Only relatively small drops in the spray in either case are obtained, and these show great uniformity, the variation in size being inside of rather narrow limits.

The new type of nozzle is the form in which the spray is in a plane inclined at the angle of  $45^\circ$  from the plane of the impinging streams, but between that and the usual style, having the spray in a plane  $90^\circ$  from that of the streams, there is the possibility of any number of intermediate forms that present any desired degree of uniformity in the size of the drops. Should a compromise nozzle giving a driving spray with greater uniformity than in the existing nozzles be desired, it can readily be constructed. The same could be secured by a disproportion between the sizes of the two streams, and in this case the coarser portion would be at one edge instead of at the center of the fan. This form might be desirable for some spot-spraying for scale insects, and it might be desirable to have a means of controlling the size of one of the streams.

• WHERE THE NEW NOZZLES ARE IMPRACTICAL

Because the spray must first be separated into two streams in this type of nozzle it becomes particularly liable to clogging and should not be used for any spraying where there is any such tendency—e. g., with Bordeaux mixture.

Most of the spray materials now used, however, are clear solutions and give no trouble in the nozzle.

LONG- AND SHORT-DISTANCE NOZZLES

When the angle is widest between the impinging streams, the angle of the fan is likewise widest, the drops finest, and the carrying distance of the spray the shortest.

An acute angle between the impinging streams produces a very narrow spray which carries a longer distance, but may perhaps finally reach nearly as great a width as that of the rapidly spreading short-distance spray.

Some prefer a long-distance nozzle and use it close to an object, as where spot spraying on a tree trunk is desired. The new type of nozzle lends itself very readily to adjustment to any degree of distance, from the shortest to nearly the longest found in spray nozzles.

ADJUSTMENT

Any form of two-stream nozzle, like that known as the calla, or lily, nozzles, can be quickly converted into a nozzle of the new type by the use of a reamer, slightly enlarging the two apertures on opposite sides by working the instrument obliquely to the surface of the nozzle and trying it from time to time until the spray sheet stands at  $45^{\circ}$ .

The same process will enable one to adjust a nozzle at any time should it wear irregularly enough to change the angle of the spray fan. The shape of the fan is a good index of the correct adjustment. If the angle is just right, the fan is triangular; if less than  $45^{\circ}$ , it is shortest in the center and the spray is coarser at the ends. If the angle is more than  $45^{\circ}$ , the fan is longest in the center and the spray coarsest at this point.

With care the reamer can be so used as to effect the change in the stream without enlarging the hole at the surface, and, therefore, not changing the volume of discharge. It may be possible to change the angle of the spread of the fan by reaming out beneath on the side adjacent to or opposite the other hole. One should continually try a nozzle while adjusting it, so as not to carry the work too far.

SUMMARY

(1) A new principle employed in nozzle construction will produce a flat spray with the qualities of a cyclone nozzle.

(2) A uniform sheet of water breaking along its edge produces drops of uniform size.

(3) A flat spray is more easily directed and produces a more uniform distribution than the cone of spray from a cyclone nozzle.

(4) Uniformly fine drops of spray aid in securing uniformity of distribution.

(5) The new nozzle allows some variation in size of spray.

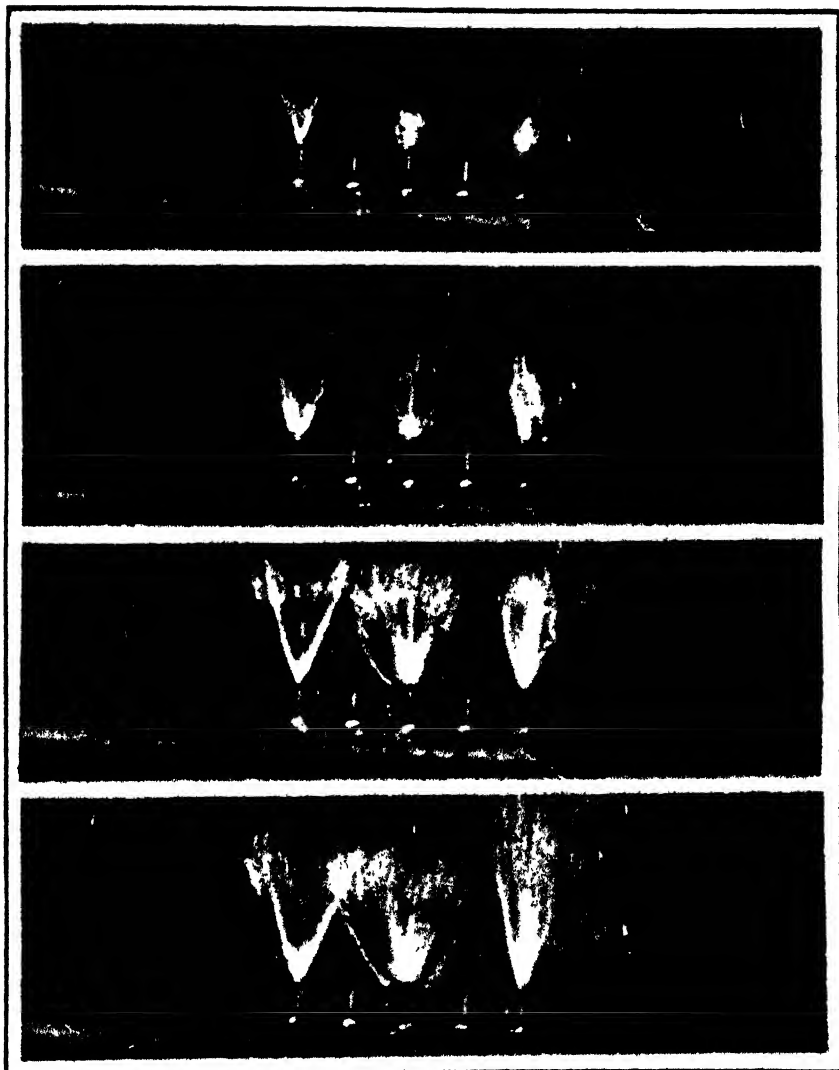
(6) It also may be made into a long- or short-distance nozzle.

(7) It can be easily constructed by modifying existing nozzles and may be adjusted if it becomes worn.



**PLATE LXXXV**

**The beginning of the spray from three kinds of nozzles, as photographed with a moving-picture camera.**



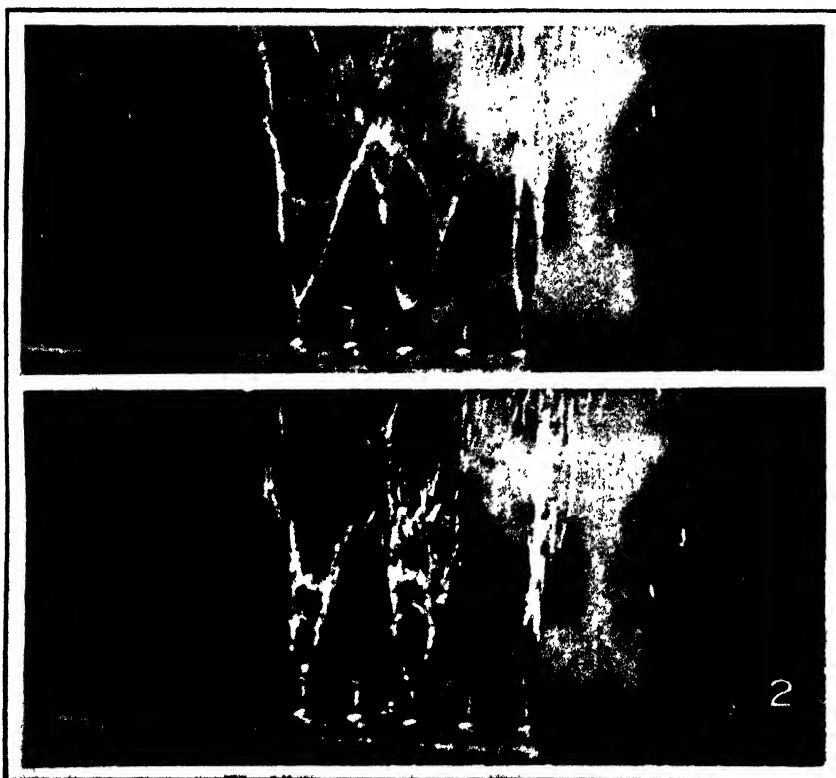
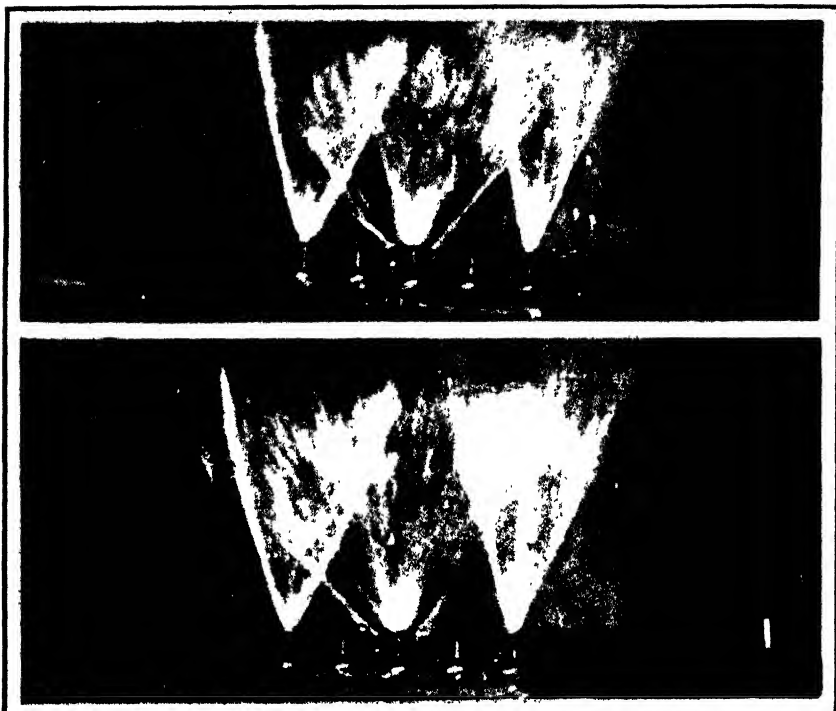




PLATE LXXXVI

Fig. 1.—The appearance of spray from three kinds of nozzles as full pressure is applied (a continuation of Plate LXXXV).

Fig. 2.—Two stages at the end of the spray as the pressure is reduced.



# A NEW INTERPRETATION OF THE RELATIONSHIPS OF TEMPERATURE AND HUMIDITY TO INSECT DEVELOPMENT

By W. DWIGHT PIERCE,

*Entomological Assistant, Southern Field Crop Insect Investigations, Bureau of Entomology*

## INTRODUCTION

Upon the proper interpretation of the laws of climatic control of life rests the solution of many practical problems, and inasmuch as all plant and animal life reacts to climate in the same general manner it is apparent that the study of the climatic control of insect development may throw light upon the problems of all other forms of life. It has been apparent to some workers in the field of ecology that our so-called laws of effective temperature were deficient in many respects. A large number of phenomena were not properly explained by any known theory. It is with the hope that the present interpretation may come closer to the truth that this paper has been prepared.

Biologists for years were laboring with the theory of a fixed zero of effective temperature for all life, and only recently was it accepted that each species might have a different zero. It has been the custom to determine the thermal constant for any given activity by multiplying the number of effective degrees accumulated above the effective zero in daily units of mean temperature by the time in which the given phenomenon took place. The noneffective low temperatures were eliminated, but not the time in which they were experienced. Inasmuch as most workers were located in north-temperate climates, where high noneffective temperatures seldom occur, it had not occurred to them that some high temperatures might not be effective and that there was another boundary to the effective zone besides the zero. These high temperatures and the time in which they are experienced must be eliminated. In addition to all of these errors in method, there has been no correlation of the humidity factor until very recently, although now many workers are trying to solve the part played by this factor.

The principal data upon which the writer has based his studies include records of thousands of individual boll weevils (*Anthonomus grandis* Boh. and *A. g. thurberiae* Pierce), made by the members of the boll-weevil force under the direction of Mr. W. D. Hunter and the writer at various localities in Texas, Louisiana, and Arizona throughout the period of years from 1902 to 1915. At each place where biological notes were made a thermograph-hygrograph record was kept, and this record was

checked twice daily by maximum and minimum thermometer and sling-psychrometer readings. The means of temperature and humidity are based upon these records. In addition to the natural records, a series of artificial-cold experiments were conducted at various times, and the writer recently conducted an extensive series of artificial-heat experiments with definite humidity control in order to determine the effects of heat.

#### EXPERIMENTAL METHODS

Before venturing to present this new interpretation the writer has thoroughly discussed it with many prominent workers, and it is now proposed for more extensive criticism and elaboration.

To express the relationship of the two factors, temperature and humidity, to insect metabolism, development, and activity, a temperature scale may be marked off on the vertical line of a sheet of plotting paper and a humidity scale from left to right on the horizontal line. There are, for any given insect, definite boundaries of atmospheric temperature and humidity within which the life of the species revolves. There is a temperature below which, even for the shortest time, life is impossible—the absolute minimum fatal temperature. There is also a temperature above which, even for a moment, life is impossible—the absolute maximum fatal temperature. Absolute dryness is more or less prohibitive of life and so is absolute humidity, or saturation, although some insects may be adapted better to withstand extremes of humidity than others. It is quite possible that the boundaries of humidity may be 0 and 100 per cent, or infinitesimally close thereto.

The diagrammatic figure sought, however, has four definite absolute boundaries—the maximum and minimum temperatures and humidities.

Within the limits which we have thus defined there exist conditions under which all the activities of the species reach their maximum efficiency. It has been conceived by most writers that this maximum efficiency was reached at a definite point known as the optimum. It seems more likely that it will prove to be a zone of humidities and temperatures of more or less restricted area. A careful study of the records of any species, charting for the time required for each activity and the temperature and then similarly for humidity, will disclose temperature and humidity points of maximum efficiency. With the boll weevil these points lie approximately near 83° F. and 65 per cent of relative humidity.

#### ZONES OF CLIMATIC RELATIONS

At any ordinary humidity, starting with the absolute minimum fatal temperature, as the temperature increases a longer and longer time of exposure is required to kill, until a point is reached at which life continues indefinitely. This zone of temperatures has been called the zone of fatal temperatures.

As the temperature continues to rise it passes through a zone of ineffective temperatures, known commonly as the zone of hibernation, which the writer will shortly prove to be an inappropriate term. At the lowest temperatures in this zone complete dormancy without metabolism is found; but as the temperature increases a gradual approach to sensibility is noted, first metabolism, next movement, and then the necessity of feeding. The point at which metabolism or growth begins at a given humidity is the zero of effective temperature.

As the temperature increases above this zero the activity is at first very sluggish, but becomes more and more active until the so-called optimum is reached, and from this point upward the temperatures cause less and less activity, inducing stupor and finally sleep or coma.

At the point of coma begins the zone of ineffective temperatures formerly known as estivation. With the increase of temperature sleep becomes more and more sound until a point is reached at which death occurs after long exposure. At this point begins the zone of high fatal temperatures at which death occurs at shorter and shorter periods until it is instantaneous at the absolute maximum fatal temperature. This completes the vertical cross section of the figure desired. A statement regarding these vertical zones was first published by the Bureau of Entomology in 1912.<sup>1</sup>

In the past, however, the fact that a similar horizontal cross section at any temperature can be made, starting at absolute dryness and reading toward absolute humidity, has not been recognized. In this manner are shown zones of fatal dryness, dryness causing stupor, increasingly effective humidity, the most effective humidity, decreasingly effective humidity, excessive humidity causing drowsiness, and finally fatal humidity, at least under certain conditions of exposure.

In the case of the boll weevil the resulting figure is a series of concentric ellipses centered about the optimum and with diagonal axes. On the accompanying diagram the main details of the relations of temperature and humidity to the boll weevil are brought out. Only a few of the more salient records are included. The development in buds (cotton squares) is based upon hundreds of individual records, but is not reported in detail. The outer lines are much less definitely located than the inner ones, but whatever their actual location the picture would be substantially the same.

#### EFFECTIVE TEMPERATURE

Workers who have used the zero of effective temperature in their studies will note that, according to the present theory, the zero when charted is an elliptical curve representing a different point at each degree

<sup>1</sup> Hunter, W. D., and Pierce, W. D. Mexican cotton-boll weevil. 62d Cong., 2d Sess., Sen. Doc. 305 (U. S. Dept. Agr. Bur. Ent. Bul. 114), p. 125-128. 1912.



of humidity. Because of the difficulty of computing this zero, the writer has been requested to describe his method of computing effective temperatures.

The first step is to tabulate all records of a given mean percentage of humidity on a single sheet. The zone of effective temperatures must be worked out separately at each degree of humidity. Only by a laborious series of testings can the first zero be approximated, unless the worker finds it by a fortunate chance. The total effective temperature is the criterion by which we finally know when we have rightly defined the limits of the zone. This is known as the thermal constant and is the multiple of the mean of the effective temperatures (between the zero and the absolute), figured in day units, by the time in which these effective temperatures were experienced. Noneffective temperatures, whether high or low, and the time in which they were experienced must be eliminated. The zone of effective temperatures will be finally reached for any given humidity when the

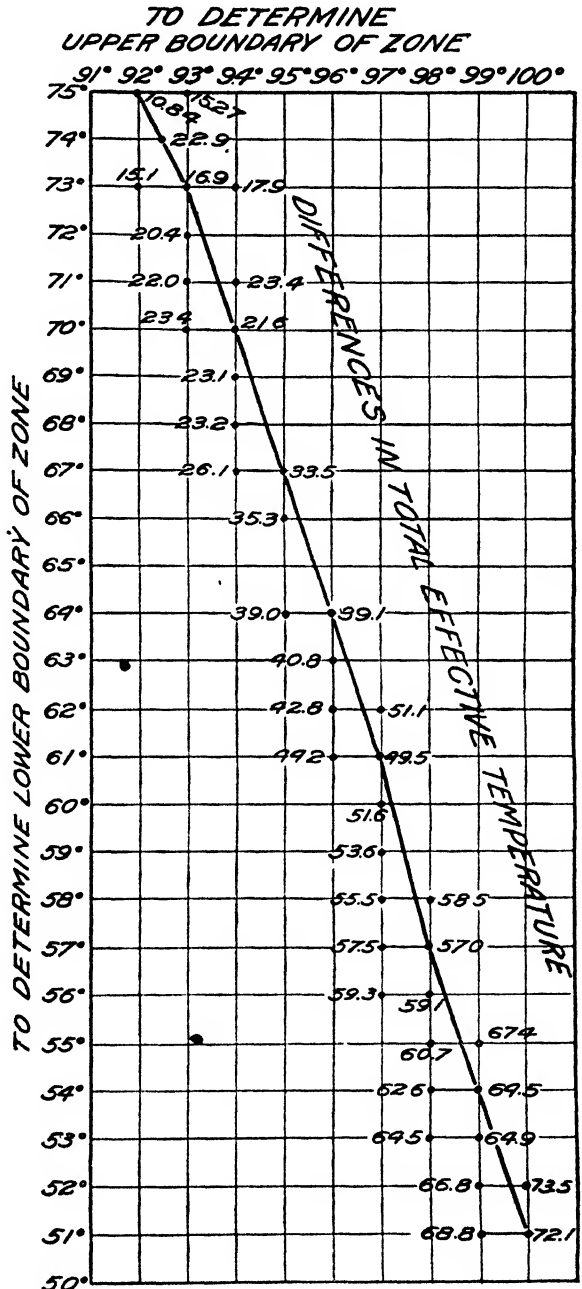


FIG. 2.—Graph showing the method of determining the zone of effective temperatures at a humidity of 56 per cent.

difference in the total effective temperatures is reduced to a minimum. At the start some arbitrary zero must be chosen and the effective temperatures computed above this. Then it is necessary to remove degree by degree at the top or bottom and note each time whether the difference in the total effective temperatures becomes larger or smaller. This process may be charted so that the general tendency can be seen. The figures found in the writer's attempt to establish the zone of effective temperatures for the boll weevil at 56 per cent humidity will illustrate the manner in which the points desired were ascertained. These results are presented in figure 2, and it will be seen that the first tentative zone chosen was 51° to 100° F. By much testing it was narrowed to within the limits of 75° to 92° F., for which the optimum is practically 83.5°.

Having obtained the limits of the zone, the records of development in cotton squares at a mean humidity of 55.9 per cent to 56.9 per cent, made at Victoria, Tex., in 1913, by Mr. B. R. Coad, of the Bureau of Entomology, are as shown in Tables I and II.

TABLE I.—Records of development of *Anthonomus grandis* at Victoria, Tex., in 1913, at a humidity of 55.9 to 56.9 per cent

Experiment.	Mean humidity.	Date of oviposition.	Time of maturing.	Actual period of development.	Number of weevils observed.		Total weevil days.	Actual temperature.		
					Male.	Female.		Absolute maximum.	Absolute minimum.	Mean.
	<i>Per cent.</i>			<i>Days.</i>				<i>° F.</i>	<i>° F.</i>	<i>° F.</i>
1.....	56.1	July 27	Aug. 9	13	6	2	104	104	73.2	88.2
2.....	56.4	July 26	Aug. 8	15	1	3	52	104	73.2	88.2
3.....	56.6	July 27	Aug. 10	14	.....	1	14	104	73.2	88.2
4.....	56.9	July 27	Aug. 11	15	.....	1	15	104	73.2	88.2
5.....	55.9	May 22	June 7	16	1	.....	16	95.5	54.5	78.2
Mean....	56.2	.....	.....	.....	Total. 8	7	201	.....	.....	.....

TABLE II.—Records of development of *Anthonomus grandis* at Victoria, Tex., in 1913, in the zone of effective temperatures, 75° to 92° F.

1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>a</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>	9 <sup>a</sup>	10 <sup>a</sup>	11 <sup>a</sup>
Experiment.	Number of weevils.	Mean humidity.	Humid time units.	Period experiment effective temperature.	Total effective weevil days.	Mean effective temperature.	Effective thermal units.	Mean daily effective temperature units.	Total effective temperature.	Humidity plus effective temperature.
		<i>Per ct.</i>		<i>Days.</i>		<i>° F.</i>			<i>° F.</i>	<i>° F.</i>
1.....	8	56.1	448.8	8.19	65.52	83.8	670.4	8.8	72.0	139.9
2.....	4	56.4	225.6	8.18	32.72	83.8	335.2	8.8	71.98	140.2
3.....	1	56.6	56.6	8.86	8.86	83.6	83.6	8.6	76.1	140.2
4.....	1	56.9	56.9	9.52	9.52	83.7	83.7	8.7	82.82	140.6
5.....	1	55.9	55.9	9.95	9.95	82.6	82.6	7.6	75.6	138.5
Total....	15	.....	843.8	.....	126.57	.....	1,255.5	.....	.....	.....
Average.	.....	56.2	.....	8.43	.....	83.7	.....	8.7	73.3	139.9
Difference.	.....	.....	.....	.....	.....	.....	.....	1.2	20.84	2.1

<sup>a</sup> Column 4 is product of columns 2 and 3. Column 5 is computed from the actual records. Column 6 is the product of 2 and 5. Column 8 is the product of 2 and 7. Column 9 is 7 minus the zero (75° F.). Column 10 is the product of columns 5 and 9. Column 11 is the sum of columns 3 and 7.



From these tables it will be seen that the effective period of development is from 8 to 10 days, averaging 8.43 days, while the actual development ranged from 13 to 16 days. It is noticeable that in all of the records the maximum as well as the minimum temperatures ran outside of the zone of effective temperatures. The total effective temperature ranged from 72° to 83° F., with 73.3° as the weighted mean and with a total difference of only 10.84°, a very small difference.

It is not necessary in this paper to give the further details of the zone of effective temperatures at other humidities. The determination of the zone for the next percentage of humidity is much less difficult, because it must be just a little narrower or a little wider than already determined. As the axis is diagonal, the upper and lower bounds will depart at a different rate. After several points have been determined, the axis can be located and then the figuring becomes very simple. It must be noted that every hour of effective temperature has its cumulative effect, even in the winter time.

#### ZONE OF INACTIVITY

One of the results of the acceptance of the present interpretation will be the necessity of discarding the conception of separate zones of hibernation and estivation. Physiologists have demonstrated that the effects of heat and cold on metabolism are alike. The writer has frequently noticed in field work the impossibility of differentiating between a frozen and a heat-killed boll-weevil larva. Prof. G. G. Becker, of Arkansas Agricultural College, several years ago observed that the fall army worm, *Laphygma frugiperda* S. and A., had two periods of activity and two of inactivity every day in the hot days in the Ozarks. Activity began in the morning and continued until the early part of the afternoon, when the heat caused the worms to be inactive for several hours. They then again became active during the early hours of the night, but the nights were cold and the worms became inactive until morning. The phenomena of a year were reproduced day by day. Inactivity due to cold in the summer time can not properly be called hibernation.

In Arizona the boll weevil is now native on wild cotton (*Thurberia thespesioides*). It normally breeds in the bolls in the fall, becoming adult by December 1, but remains in its cell throughout the cold winter and the warming spring. In some canyons there is a spring rainy season and *T. thespesioides* has a spring fruiting season. In these localities the moisture also releases the weevils from their cells and they begin breeding. A dry season follows and the weevils go to sleep. In other canyons the spring is not wet and the plants and weevils are inactive until the regular rainy season in August, when the long rest is broken. In some canyons the weevils therefore have two resting periods during the year, and in other canyons they are at rest from fall until summer. It not infrequently happens that the August rainy season does not materialize, and under

such circumstances the weevils stay in their cells and the plants remain dormant until the next year or perhaps for several years. As evidence of this, the writer kept several of these weevils over 500 days without food or water, and one lived 626 days, dying only when moisture invaded the room where it was kept.

Hunter, Pratt, and Mitchell<sup>1</sup> record the unusual ability of larvæ of *Hermetia chrysopila* Loew, a cactus scavenger fly, to withstand long periods of drought. Larvæ in various stages of development were kept for more than 15 months without food and developed readily later when food was supplied. The very leathery integument seems to protect the insect against desiccation, and in other ways the larva has evidently adapted itself to long periods of waiting for favorable food, which, in the arid regions, depends upon the infrequent rains. Both of these instances are more properly resting periods due to dryness than to cold or heat.

#### NOMENCLATURE OF CLIMATIC EFFECTS ON LIFE

As charted, there are three elliptical zones which express the three principal effects of climate on life, viz, activity, inactivity, and death. The zone of activity may be known as the "thermopractic" zone (θερμός, meaning heat, plus πρακτικός, meaning effective). The zone of inactivity may be known as the zone of "anesthesia" (άναισθησία, meaning insensibility). The zone of death may be known as the "olethric" zone (όλέθριος, meaning deadly). The region of greatest activity may be known as the "practicotatum" zone (πρακτικώτατον, meaning most effective).

Many phases of climatic effects have been differentiated, and medical literature abounds in words descriptive of these effects. For some effects no words are available. The writer has thought it best to present a complete and consistent system of nomenclature, based on the Greek, using all words already in the language, and only supplying new words where none are now available.<sup>2</sup>

It may be convenient to refer to the most effective temperature or the most effective humidity, in which cases we may use the words "thermopracticotatum" or "hygropracticotatum."

The awakening from sleep is termed "anastasis" (άνάστασις). We can therefore speak of "thermanastasis" and "hygranastasis," depending on whether the awakening is caused by a change of temperature or a change of humidity.

Heat, moisture, dryness, or cold added to the "practicotatum" will cause sluggishness. We have to indicate this condition the term "nochelia"

<sup>1</sup> Hunter, W. D., Pratt, F. C., and Mitchell, J. D. The principal cactus insects of the United States, U. S. Dept. Agr. Bur. Ent. Bul. 113, p. 38-39. 1912.

<sup>2</sup> New Standard Dictionary. 1913.

Gould, G. M. An Illustrated Dictionary of Medicine, Biology and Allied Sciences . . . ed. 6, with . . . Sup . . . 1633, 571 p., Philadelphia, 1910.

(νωχέλεια, meaning sluggishness) and can show the type of sluggishness by the addition of a prefix, as "thermonochelia," "hygronochelia," "xeronochelia," and "rhigonochelia."

At least three of these factors produce under extreme conditions a stifling sensation, and we may express this by the terms "thermopnigia," "xeropnigia," and "hygropnigia" (πνίγος, meaning stifling).

The stifling sensation ends in complete insensibility, or anesthesia, and this word may be modified to express the cause, as in the term "thermanesthesia," "hygranesthesia," "xeranesthesia," and "rhiganesthesia."

Death from heat is known as thermoplegia (πληγή, meaning stroke), while from excessive moisture it may be known as "hygroplegia," and from freezing, as "rhigoplegia." Death from drying is known as "apoxeraenosis" (ἀποξηραίνω, meaning to dry up).

The determination of locomotion by heat is called "thermotaxis," and movement brought about by heat is called "thermotropism."

Unusual sensibility to heat is called "thermalgesia" and "hyperthermalgesia." The ability to recognize changes of temperature is "thermesthesia," and its extreme is designated as "thermohyperesthesia," abnormal sensitiveness to heat "stimuli." Fondness for heat or requiring great heat for growth is called "thermophilic," while resistance to heat is called "thermophylic." Rapid breathing, owing to high temperature, is designated as "thermopolypnea," contraction under the action of heat as "thermosystaltic," adapting the bodily temperature to that of the environment as "pecilothermal," and a morbid dread of heat as "thermophobia."

The life after apparent death, called "anabiosis," is exemplified in such cases as that of the *Hermetia* larvæ mentioned above.

Pain from the application of cold is called "cryalgnesia," abnormal sensitiveness to cold "cryesthesia," and a morbid sensitiveness to cold "hypercryalgnesia."

#### PRACTICAL APPLICATIONS

Many practical measures will result from the further study of climatic relations to life. A few of these may be indicated.

One of the most effective measures for the control of the cattle tick is pasture rotation based upon the possible duration of life of the seed tick without an animal host. As this period varies with the season, it is necessary to know the climatic laws under which this species reacts.

The fall army worm advances across the country and again retreats in complete accord with changing temperatures. The proper fixation of the zone of effective temperature may make it possible to plan the planting of winter crops to avoid damage.

The cotton boll weevil must have food up to the time that it enters hibernation. Early harvesting and destruction of stalks before the low temperatures set in offer one of the most satisfactory methods of control.

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